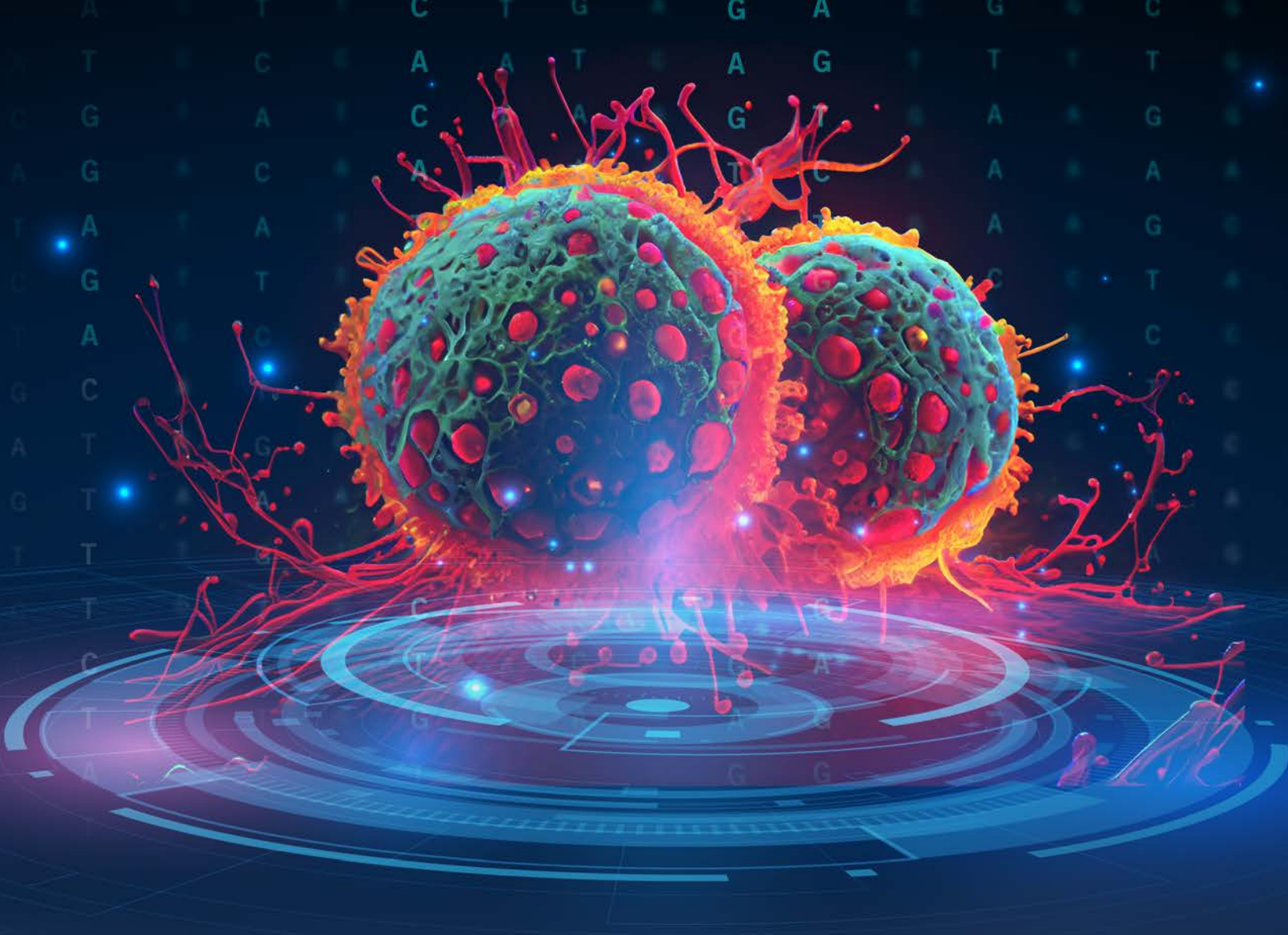

SALK CANCER DAY SYMPOSIUM 2024

ABSTRACT BOOK

TECHNOLOGY REVOLUTIONIZING CANCER RESEARCH

FRIDAY, NOVEMBER 1, 2024

salk® Institute for Biological Studies

Abstracts of papers presented at

Salk Cancer Day Symposium

November 1, 2024

Organized by Salk Institute Researchers:

Corey Jones-Weinert
Louis Parham
Shira Yomtoubian
Diana Hargreaves
Susan Kaech
Geoffrey Wahl

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University of California San Diego

An Ex-Vivo Organotypic Culture Platform of Mucinous Carcinomatosis Peritonei Identifies CDK4/6 Inhibition as a Novel Treatment

POSTER ABSTRACTS

Cell Mechanics Innovations in Oncology: Harnessing Biophysical Principles for Cancer Intervention

Roberto Alonso Matilla[1], Teemu P. Miettinen[2], Paolo P. Provenzano[1], and David J. Odde[1]
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T cell migration: Despite recent progress in understanding amoeboid-mesenchymal migratory balance, it remains largely unknown how T cells mechanically move through tumors and what factors set their migration capabilities. To address this, we have developed a biophysical T cell migration model that elucidates the potential physical principles and molecular components modulating their movement. The model results are complemented by preliminary data obtained from *in vitro* T cell migration studies. We first examined the potential for adhesion-free bleb-based migration and show that cells only inefficiently migrate in the absence of adhesion-based forces, i.e., cell swimming. However, our model suggests that T cells can employ a hybrid bleb- and adhesion-based migration mechanism for rapid cell motility and identifies conditions for optimality [1].

Leukemia cell cytokinesis: The impact of actomyosin forces on the plasma membrane during cytokinesis is poorly understood. By using a combination of imaging and biophysical modeling, we found an extensive accumulation and folding of the plasma membrane at the cleavage furrow and the intercellular bridge [2]. This is caused by actomyosin pulling the plasma membrane toward the cleavage furrow and by local cell surface area changes driven by the radial constriction of the furrow. Our work reveals that actomyosin-based mechanisms responsible for cytokinesis can also decrease membrane tension at the intercellular bridge, potentially promoting cytokinetic fidelity and locally altering endocytosis, exocytosis, and cell signaling.

References
[1] bioRxiv, https://www.biorxiv.org/content/10.1101/2023.10.29.564655v3
[2] R. Alonso-Matilla, A. Lam, T. P. Miettinen, “Cell intrinsic mechanical regulation of plasma membrane accumulation in the cytokinetic furrow”. PNAS (2024)

Multiplexed ORF Expression Screens Unveil Determinants of Adverse Prognosis in AML

Nahal Azimi, Anagha Deshpande, and Ani Deshpande
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Despite therapeutic advancements, still a significant percentage of pediatric acute myeloid leukemia patients experience recurrence or primary induction failure. Leukemic stem cells (LSCs) are crucial drivers of leukemia relapse and resistance to therapy. A 47-gene signature that is highly expressed in LSCs (LSC47) has been identified as a strong predictor of dismal prognosis in pediatric AML. Despite the role of these stem cell-associated genes in AML progression, their contribution in leukemogenesis and/or therapy resistance remains undefined. We hypothesize that each of the LSC47 genes contributes to leukemia pathogenesis in distinct ways and to test this hypothesis, we will overexpress each LSC47 gene in AML cells in a pooled format and subject cells to diverse selection pressures to identify the top gene candidates that enhance the AML cell fitness under each pressure. This approach will allow us to characterize LSC47 signature genes more thoroughly under heterogenous conditions using a high-throughput platform and provide a more complete understanding of the contribution of these genes to leukemia maintenance and therapeutic resistance. The goal is to identify novel regulators of AML cell fitness and investigate for novel mechanisms of drug resistance.

Pathways and Mechanisms of Drug Resistance in HIV

Avik Biswas[1,2], Indrani Choudhuri[1,3,4], Min Li[5], Dario Passos[1], Zelin Shan[1], Allan Haldane[3,6], Robert Craigie[5], Ronald M. Levy[3,4,6], and Dmitry Lyumkis[1,7]
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Drug resistance to antiretroviral therapy remains a pervasive problem in the treatment of HIV/AIDS. We use a novel methodology combining a Potts sequence-covariation statistical-energy model of HIV protein fitness under drug selection pressure, with kinetic Monte Carlo simulations of sequence evolutionary trajectories, to explore the temporal acquisition of drug resistance in patients as it arises in an ensemble of drug-naive HIV patient protein sequences. Our simulations accurately capture the (clinically) reported time to acquire DRMs across the major HIV-1 drug target enzymes, protease, reverse transcriptase, and integrase; slowly acquired DRMs are contingent on a network of epistatic interactions with accessory mutations that appear only after prolonged drug pressure. This result highlights the central role of epistasis in determining the kinetics governing DRM emergence. We focus our mechanistic understanding of drug resistance on the integrase enzyme, which is targeted by the integrase strand transfer inhibitors (INSTIs). For the slowest acquired DRMs against INSTIs, we define the temporal ordering of events along mutational pathways leading to drug resistance starting from specific, wild- type laboratory molecular clones of HIV, making use of the Potts model's ability to effectively predict mutational likelihoods in the evolving sequence background. We then rationalize and provide the mechanistic bases for the preferred pathways to drug resistance by ordering the mutant structures derived by high-resolution cryo-electron microscopy (cryo-EM) along the predicted trajectories. This work provides a framework for the development of combined computational and structural biology approaches to surveil the response of systems including cancer to external selection pressure from therapeutics, rationalize the mechanisms of resistance, and elucidate potential opportunities for therapeutic interventions.

Investigating the Ins and Outs of Telomere-mediated Proliferative Boundaries

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Replicative immortality is a hallmark of cancer, whereas in healthy, somatic cells, replication potential is limited by telomeres, repeat sequences at the end of chromosomes that shorten with each cell division. Successive cell divisions in primary human cells results in telomeres that are too short to retain protective binding proteins and secondary structures. Deprotected telomeres are recognized by the DNA damage response (DDR), which activates either of two distinct tumor suppressor programs: replicative senescence or replicative crisis. Cellular transformation requires cells to bypass these replicative barriers by activating a telomere maintenance mechanism. This represents a fundamental distinction between the biology of cancer versus somatic cells that may be leveraged to prevent and treat cancer. Thus, the main goal of this project is to gain a deep molecular understanding of how telomeres regulate senescence and crisis and how cells bypass these boundaries to become immortal. First, to identify novel intracellular mediators of proliferative boundaries, we performed a BioID screen to explore the nuclear and telomere proteome during replicative senescence and replicative crisis. IMR90 human lung fibroblast cells expressing a biotin ligase known as BirA were grown to senescence and crisis and compared to replicatively young control cells. Biotinylated proteins were identified through mass spectrometry, and the data generated are currently being analyzed. Future studies will identify proteins that mediate senescence and crisis and investigate their role in initiation and bypass of these proliferative boundaries. In addition to this screen, we began to examine extracellular regulation of proliferative boundaries. This is because senescent cells are characterized by the senescence-associated secretory phenotype (SASP), which has autocrine and paracrine effects that enforce and transmit senescence and contributes to age-related diseases including cancer. In contrast to senescence, a secretory phenotype of crisis cells has not yet been identified. However, preliminary evidence from our lab suggests that cell death in crisis is mediated through the actions of a crisis-associated secretory phenotype (CASP). To begin to explore the role of the CASP, young cells were treated with conditioned media from either growing cells or crisis cells. Remarkably, after just 48 hrs of exposure, crisis cell conditioned media upregulated numerous interferon-stimulated genes in young cells. Future studies will determine if the CASP drives cell death in crisis, identify its receptors and effectors, and characterize its molecular constituents. In total, these studies will identify novel intra- and extra-cellular regulators of telomere-mediated proliferative boundaries.

Coordination of GPCR-mediated Hippo Pathway Signaling by ARRDC3 and Src Family Kinases in Invasive Breast Carcinoma

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G protein-coupled receptors (GPCRs) are implicated in cancer progression. In particular, overexpression of the GPCR protease-activated receptor-1 (PAR1) has been linked to poor patient prognosis and metastasis of breast carcinoma. However, there remains a critical gap in knowledge of how PAR1 expression leads to breast cancer progression, specifically the deregulation of downstream signaling pathways such as the Hippo pathway. The long-term goal of this project is to determine the GPCR-mediated signaling networks that control breast cancer progression and metastasis. The overall objective of this study is to understand the interplay between Src family kinases (SFKs) and the α-arrestin ARRDC3 and determine how this impacts Hippo pathway signaling to promote breast cancer progression and metastasis. We hypothesize that the SFK-ARRDC3 interaction functions as a key regulator of GPCR-mediated Hippo signaling in breast cancer and thus contributes to cancer progression and metastasis. This interaction will be further explored via cancer-based assays both *in vitro* and *in vivo* to assess whether SFK-mediated YAP-TAZ activation drives tumor growth and metastasis. The proposed work is conceptually innovative because it will reveal a previously unexplored mechanism by which SFKs and ARRDC3 intersect to drive tumor metastasis and progression via GPCR-stimulated Hippo pathway signaling. The proposed research is significant because it will define a fundamental mechanism to explain breast carcinoma progression in the context of GPCRs and Hippo pathway signaling and further promote the discovery of new therapeutic targets that are vital components of the pathway and may lead to the development of new and effective treatments.

KATs on a Leash – Targeting Oncogenic KAT2 Activities in AML

Anagha Deshpande[1], Marlenne Perales[1], Aditi Pedgaonkar[1], Neha Niranjan[1], Pramod Akula Bala[2], Rabi Murad[2], Darren Finley, Kristina Vuori, and Ani Deshpande[1]

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Several leukemia-associated oncoproteins aberrantly activate transcriptional circuits resembling a stem-like state in Acute Myeloid Leukemia (AML). This activation of "stemness" genes is achieved by enlisting the activity of specialized components of the epigenetic machinery. These epigenetic regulators sustain the transcriptional memory of transformed cells with aberrant self-renewal properties and the inability to undergo terminal myeloid differentiation. Using the dependency maps (DepMap) dataset, we sought to identify protein complexes that are most highly selectively essential in AML cells compared to cancers of other lineages. This analysis revealed that the SAGA complex was one of the most AML-selective dependencies in the entire DepMap dataset, predicting that SAGA complex inhibition will have selective activity in AML compared to other tumors. Indeed, experiments using GSK983, a proteolysis targeting chimera (PROTAC) targeting KAT2A and B, the key histone acetyltransferases in the SAGA complex on ~200 cell lines of diverse lineages revealed that AML cell lines were amongst the most highly sensitive compared to other lineage cancers. Furthermore, GSK983 treatment significantly reduced the proliferation of AML cell lines of diverse mutational profiles, and of patient derived cells from treatment resistant AML. Of note, GSK983 also showed a strong synergy with several standard-of-care anti-AML therapies including cytarabine, doxorubicin and revumenib. Moreover, GSK983 treatment led to a significant loss of self-renewal associated genes, including *HOXA13* and *MYC* at the transcriptional levels as measured using RNA-seq as well as protein levels as assessed using mass spectrometry. GSK983 treatment was accompanied by an increased differentiation of AML cells, and a concomitant induction of apoptosis and cell cycle arrest. Intriguingly, GSK983 treatment led to the increase of several interferon stimulated genes (ISGs) as assessed using metabolic labeling of RNA (SLAM-seq) and proteomics (Mass Spec). Early timepoint SLAM-seq results showed that MYC downregulation and ISG upregulation were immediate consequences of KAT2A/B degradation. These results prompted us to test if ISG activation enhances AML cell killing by immune cells. Interestingly, our preliminary studies showed that GSK983 treatment significantly augments T-cell killing of human AML cells while also independently promoting T cell activation. Ongoing studies are now focused on assessing the role of KAT2/B in suppressing the anti AML immune response.

Resistance to Autophagy Inhibitors Causes Rewiring of Pyrimidine Metabolism in PDAC and Increases Sensitivity to Pyrimidine Analogs

Suzanne Dufresne[1], Anvita Komarla[1,2], Ramya Kuna[1], Adelaida Estrada- Cardenas[1,3], Payel Mondal[1], Louis Parham[1], Kristiana Wong[1,3], Angelica Rock[1,3], Celina Shen[1,3], Joel Encarnación Rosado[1], Amanda Cyril[1,3], Alva Sainz[1], Gerald Shadel[1], Dannielle Engle[4], Christian Metallo[1], and Christina Towers[1]

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Autophagy is a cellular recycling process that is increased in pancreatic ductal adenocarcinoma (PDAC) and has recently emerged as a promising therapeutic target for this disease. This catabolic process degrades and recycles cellular material to ensure protein turnover, organelle quality control, and metabolic homeostasis. For example, autophagy breaks down nucleic acids to provide nucleotide building blocks which may allow cancer cells to maintain their high proliferation rates even in harsh environments. Based on several pre-clinical studies showing decreased tumor growth in autophagy-deficient mice, autophagy inhibitors are being tested in clinical trials. However, the results are mitigated, and resistance mechanisms are likely but remain largely uninvestigated. My project aims to identify how cancer cells can become resistant to autophagy inhibition to propose new treatment strategies. I generated PDAC cells resistant to the autophagy inhibitor hydroxychloroquine (HCQ) or MRT68921 (MRT) by exposing them to increasing doses of these drugs. Our group previously generated cancer cell lines resistant to genetic inhibition of autophagy and recent findings show they acquire dependency on pyrimidine metabolism. Based on these results, I performed targeted metabolomics to determine the alterations in pyrimidine metabolism in these cells. I found that the drug-resistant cells have decreased pyrimidine pools. I also found a buildup of aspartate and reduced levels of intracellular monophosphate nucleotides suggesting that the cells have blocked de novo pyrimidine synthesis. Moreover, I found decreased gene and protein expression of the rate limiting enzyme for de novo pyrimidine synthesis, DHODH, in the drug-resistant cells. In contrast, the expression of genes implicated in the salvage of pyrimidines was increased in HCQ- and MRT-resistant cells when compared to the controls. Interestingly, pyrimidine analogs are common chemotherapeutic agents used in PDAC and are taken by cells via the pyrimidine salvage route. Using cell viability assays, I found that HCQ- and MRT-resistant cells have increased sensitivity to the pyrimidine analogs gemcitabine and trifluridine-tipiracil. I now aim to investigate whether the combination of autophagy inhibitors and pyrimidine analogs represents an effective strategy in PDAC.

Discovery and Characterization of Novel ATG13 Degrading Compounds to Inhibit Autophagy and Treat Lung Cancer

Patrick Martin Hagan, Allison Limpert, Douglas Sheffler, Huiyu Ren, and Nicholas Cosford
Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA

Lung cancer is the leading cause of cancer-related mortality across all populations. Autophagy (ATG) is a cell survival process by which dysfunctional cellular components are removed and recycled to maintain homeostasis through a lysosomal dependent degradation mechanism. ATG upregulation is implicated as a bad actor in several diseases, particularly K-Ras driven non-small cell lung cancer (NSCLC), where it provides nutrients to lung cancer cells in the nutrient poor and hypoxic tumor microenvironment. ATG also contributes to chemotherapeutic resistance, making it a viable pathway for therapeutic intervention. However, the only clinically available drugs targeting ATG are chloroquine and hydroxychloroquine, which are not ATG-specific and have multiple adverse side effects. ATG is an intricate, multistep process beginning with the Unc-51-like autophagy-activating kinases 1 and 2 (ULK1/2) initiation complex. The supramolecular assembly of this complex relies on the adaptor protein ATG13, which binds ULK1/2. Knockout of ATG13 significantly reduces the viability of lung cancer cell lines, indicating that ATG13 is a viable therapeutic target for lung cancer. Furthermore, our group has characterized other autophagy inhibiting compounds that also robustly degrade ATG13 in cells and tissues, leading to cancer cell death. We have developed and optimized a high throughput screening (HTS) assay whereby ATG13 is HiBit tagged in A549 NSCLC cells (ATG13 HiBit assay) to allow identification of compounds that induce ATG13 protein loss, and therefore inhibit ATG. We have also developed a testing funnel that employs multiple assays to characterize compounds that degrade ATG13 independent of cellular toxicity (cell viability assay), confirm their activity against our protein of interest (In Cell Western blot ATG13 assay), measure their ability to inhibit autophagy (GFP-LC3-RFP-LC3ΔG ATG flux assay) and eliminate compounds that cause assay interference (HiBit counterscreen). Using these assays we have identified novel lead compounds which degrade ATG13 and inhibit autophagy resulting in cell death in NSCLC.

Targeting Protein Fatty Acylation to Inhibit Colorectal Cancer Growth

Linnea Hases[1], Sihao Liu[1], Gia H. Quach[1], Yang Dai[1], Annette R. Atkins[1], Ruth T. Yu[1], Christopher Liddle[2], Michael Downes[1], and Ronald M. Evans[1]
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Excess intake of dietary fat is a risk factor for colorectal cancer (CRC), however the mechanistic underpinnings of this association remain poorly understood. Aberrant fatty acid metabolism has long been recognized in CRC cells. While conventionally viewed as building blocks for cellular membranes and substrates for mitochondrial energy production, protein fatty acylation is also utilized in the covalent modification of hundreds of proteins. Protein fatty acylation regulates oncogenic signaling by regulating cellular localization, protein stability, protein-protein interaction, trafficking, and activity, thereby linking metabolic states to cellular functions. Among the regulators of this process, HDAC11, a lysine fatty acid deacylase, is significantly upregulated in CRC and exhibits over 10,000-fold higher catalytic efficiency for fatty acid deacylation compared to deacetylation. Our investigations utilizing both genetic knockouts and the pharmacological inhibitor SIS17 reveal that while HDAC11 is dispensable for normal organismal function, it plays a crucial role in CRC growth. To gain further insight into the mechanisms by which HDAC11 regulates CRC tumor growth, we next employed an unbiased click chemistry-based mass spectrometry screen for HDAC11 inhibitor (SIS17) sensitive protein palmitoylations (the major type of lysine fatty acid acylation). This analysis uncovered numerous receptor tyrosine kinases, important for oncogenic signaling, including HER3. Moreover, using The Cancer Dependency Map (DepMap) portal we identified that cell lines with a hotspot mutation in HER3 respond better to silencing of HDAC11. HER3 is a member of the EGFR (ErbB) family of receptor tyrosine kinases and plays a critical role in cell growth and survival. Although HER3 has low intrinsic kinase activity, it forms heterodimers with other receptors, like HER2 or EGFR, to activate downstream signaling pathways. We have found that increased palmitoylation of HER3 downstream of HDAC11 inhibition blocks EGFR-HER3 heterodimerization and HER3 membrane expression, resulting in reduced HER3/EGFR signaling and downstream MAPK activation (p-ERK1/2). Importantly, HER3 has been implicated in CRC therapeutic resistance, especially to EGFR and KRAS-targeted therapies. In line with this, we find that HDAC11 and HER3 are both induced upon KRAS inhibition, cooperatively supporting HER3 signaling in KRAS resistance. Moreover, we demonstrate that combination of SIS17 with KRAS inhibition shows improved efficacy in inhibiting CRC growth. These findings position HDAC11 as a promising therapeutic target in CRC, with the potential to improve outcomes for patients facing therapeutic resistance.

A Novel Phosphatase in Matrix Stiffness-driven EMT and Tumor Metastasis

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Mechanical cues from the extracellular matrix (ECM) regulate various cellular processes in cell proliferation, migration and differention via distinct mechanotransduction pathways. Increase in tissue stiffness is correlated with distant metastasis and poor outcome in breast cancer patients. Previous studies show that increased ECM stiffness promotes Epithelial-Mesenchymal Transition (EMT), cell invasion and metastasis. Using 3D reconstituted extracellular matrixes that recapitulate the range of physiological stiffness from normal mammary glands to breast tumors, we identified TWIST1 as a key player driving EMT and invasion in response to increasing ECM stiffness. High ECM stiffness activates the LYN kinase, which phosphorylates the EMT transcription factor TWIST1 to release TWIST1 from its cytoplasmic anchor G3BP2 to enter the nucleus, thus triggering EMT and invasion. To understand how LYN is activated at high stiffness, we have performed an unbiased screen for tyrosine phosphatases that dephosphorylate LYN on its inhibitor phosphorylation site, thus activating LYN in response to matrix stiffness. My study identified a novel receptor tyrosine phosphatase, protein tyrosine phosphatase epsilon (PTPRE) as being essential in promoting EMT and invasion at high matrix stiffness. Mechanistically, PTPRE directly interacts with LYN at high stiffness and is essential for LYN activation and TWIST1 nuclear trasnlacation at high stiffness. My ongoing work is testing the role of PTPRE in promoting invasion and metastasis in cell-derived and patient-derived breast cancer organoids and xenografts. Together, this study identified a novel role of the receptor tyrosine phosphatase PTPRE in matrix stiffness-driven EMT and breast cancer metastasis.

SUMO2 Inhibition Reverses Aberrant Epigenetic Rewiring Driven by Synovial Sarcoma Fusion Oncoproteins and Impairs Sarcomagenesis

Rema Iyer[1], Anagha Deshpande[1], Aditi Pedgaonkar[1], Pramod Akula Bala[2], Taehee Kim[3], Gerard L.Brien[4,5], Darren Finlay[6], Kristiina Vuori[6], Alice Soragni[3], Rabi Murad[2], and Aniruddha J. Deshpande[1*]

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Synovial Sarcoma (SySa) is an aggressive soft tissue sarcoma that accounts for 5 – 10% of all soft tissue sarcomas. Current treatment involves radiation and radical surgery including limb amputation, highlighting the urgent need to develop targeted therapies. We reasoned that transcriptional rewiring by the fusion protein SS18-SSX, the sole oncogenic driver in SySa, creates specific vulnerabilities that can be exploited for treatment. To uncover genes that are selectively essential for SySa, we mined The Cancer Dependency Map (DepMap) data to identify genes that specifically impact the fitness of SySa compared to other tumor cell lines. Targeted CRISPR library screening of SySa-selective candidates revealed that the small ubiquitin-like modifier 2 (SUMO2) was one of the strongest dependencies both i*n vitro* as well as *in vivo*. TAK-981, a clinical-stage small molecule SUMO2 inhibitor potently inhibited growth and colony-forming ability. Strikingly, transcriptomic studies showed that pharmacological SUMO2 inhibition with TAK-981 treatment elicited a profound reversal of a gene expression program orchestrated by SS18-SSX fusions. Of note, genetic or pharmacological SUMO2 inhibition reduced global and chromatin levels of the SS18-SSX fusion protein with a concomitant reduction in histone 2A lysine 119 ubiquitination (H2AK119ub), an epigenetic mark that plays an important role in SySa pathogenesis. Taken together, our studies identify SUMO2 as a novel, selective vulnerability in SySa. Since SUMO2 inhibitors are currently in Phase 1/2 clinical trials for other cancers, our findings present a novel avenue for targeted treatment of synovial sarcoma.

Our study identifies SUMO2 as a selective dependency in synovial sarcoma. We demonstrate that the SUMO2/3 inhibitor TAK-981 impairs sarcomagenesis and reverses the SS18-SSX fusion-driven oncotranscriptome. Our study indicates that SUMO2 inhibition may be an attractive therapeutic option in synovial sarcoma.

Understanding the Role of ARID1A in Pancreatic Ductal Adenocarcinoma

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Pancreatic Ductal Adenocarcinoma (PDAC) is one of the deadliest cancers, with a 5-year relative survival rate under 13%. Among failed treatments for PDAC is immune-checkpoint blockade (ICB); however, recent clinical meta-analyses have shown that for a variety of cancers, including PDAC, mutations in the BAF chromatin remodeling complex may promote ICB efficacy. The BAF complex is mutated in ~20% of human cancers, and the canonical BAF (cBAF) complex contains the ARID1A subunit which mutated in approximately 9% of PDAC cases. The goal of this project is to determine the function of ARID1A in PDAC progression and investigate ARID1A mutation as a biomarker for ICB responsiveness. Here, we use an *in vivo* orthotopic model combined with *in vitro* cellular studies to define the mechanisms by which cBAF mutations affect tumor growth and the tumor-immune microenvironment. Initial results show that ARID1A-deficient tumor cells have variable responses to interferon stimulation and have different proliferative capacity *in vitro*. We observe growth differences of these cells *in vivo* and changes in the tumor microenvironment. Our future work will incorporate ICB treatment and leverage single-cell transcriptomic approaches in specific immune populations (such as CD8 T cells) to determine how ARID1A mutation in tumor cells may rewire the transcriptome of other cell types to potentially allow enhanced ICB responsiveness. Determining the mechanisms behind ICB-sensitive, BAF-mutant PDAC tumors will provide a framework for understanding which pathways may be targeted to promote immune therapy responsiveness in PDAC tumors.

An Oncogenic Role for the Cohesin Complex at Structural Variants

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Structural variants represent a pervasive yet relatively under-studied class of cancer-driving mutations. While some features of cancer genomes like copy number amplification and gene fusions are understood to functionally contribute to oncogenesis and disease progression, less is known about the regulatory impact of complex rearrangements. In their native contexts, genes are insulated from ectopic enhancers by the cohesin complex, a ring-like structure that associates with chromatin to extrude loops of self-interacting genetic elements. In the case of structural variants like translocations and inversions, genes can become incorporated into novel chromatin loops that enable their ectopic activation. Since cancer genomes routinely display such complex rearrangements, these "enhancer hijacking" events are a potentially critical feature of oncogenesis. We previously engineered de novo translocations at the MYC locus in neuroblastoma cells which showed differential activation of MYC expression dependent on enhancer activity in the translocation partner region. To identify factors which might facilitate the ectopic activation of MYC by these non-native enhancers, we performed genome-wide CRISPR knockout screens in the engineered models. These experiments identified the mutually exclusive cohesin subunits STAG1 and STAG2, wherein STAG1 promoted MYC activation while STAG2 suppressed it despite their synonymous roles in the cohesin complex. Knocking out STAG2 in a MYC-activating translocation model showed further enhancement of MYC expression, while the same knockout in the parental neuroblastoma cell line had no effect. This result suggests a cancer-specific role for these subunits in regulating enhancer hijacking. Next, we found a clonally differential increase in STAG1 protein levels across the double-mutant cell lines as compared to MYC-wildtype cells with STAG2 knockout. Together, these results suggest a compensatory mechanism by STAG1 that is critical for facilitating enhancer hijacking at the novel MYC locus. Intriguingly, pan-cancer analyses show an enrichment for loss-of-function mutations in STAG2 while STAG1 is generally amplified in patient cases. We hypothesize that the STAG1-cohesin complex has a distinct capacity for generating novel chromatin loops between genes and distal enhancers, as in structural variants. To this end, we have performed Hi-C to observe both local and global changes to genome conformation following loss of STAG2, with the expectation that compensation by STAG1 will promote longer chromatin loops that can incorporate the translocated MYC allele into a new regulatory context. Performing these experiments in additional engineered translocation models and at endogenous translocations in other cancer cell lines will elucidate the generalizability of STAG1 as a positive regulator of enhancer hijacking.

Fgf1 Alleviates Cancer Cachexia by Inhibiting Beige Adipogenesis

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Cancer-associated cachexia (CAC) is a wasting syndrome characterized by weight loss, muscle atrophy, anorexia, and anemia, affecting 80% of patients with advanced cancer and directly contributing to 20% of cancer-related deaths. However, the exact mechanisms of CAC remain incompletely understood, limiting therapeutic development. Current treatments targeting CAC primarily aim to: 1) stimulate appetite to increase nutritional intake or 2) prevent muscle mass loss. However, no attempts have been made to target adipose tissue, another major organ affected by CAC, to prevent its progression. Here, we propose that fibroblast growth factor 1 (Fgf1) delays the progression of CAC by inhibiting beige adipogenesis. Recent studies have shown that browning of white adipose tissue (WAT) occurs in the early stages of cachexia and contributes to increased energy expenditure. This increased energy expenditure may contribute to a hypermetabolic state, making it difficult for patients to maintain body weight and muscle mass. Thus, inhibiting beige adipogenesis in WAT may slow or potentially alleviate the progression of cancer cachexia.We demonstrate that administering Fgf1 to mice with advanced cancer inhibits beige adipogenesis, preventing the loss of muscle function and adipose tissue mass by reducing energy expenditure, likely through the suppression of thermogenic gene expression, including Ucp1. To confirm Fgf1's role in inhibiting beige adipogenesis, we administered Fgf1 under cold-exposure conditions and observed a similar suppression of beige adipogenesis as seen in cancer cachexia mice. We show that Fgf1 disrupts the interaction between the transcription factors PPARγ and PRDM16, preventing their recruitment to thermogenic gene promoters, thereby inhibiting thermogenic gene expression and beige adipogenesis. In conclusion, Fgf1 possesses the ability to inhibit beige adipocyte differentiation, when applied in the context of the hypermetabolic state of CAC, suppresses beige adipogenesis and consequently slows the progression of CAC. This suggests that Fgf1 could be developed as a therapeutic drug to inhibit beige adipocyte differentiation and thus ameliorate CAC.

Identifying Novel Mechanisms of Quality Control in Autophagy-deficient Cancer Cells

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Autophagy is a cellular recycling process that regulates organelle quality control. A specific form of autophagy that maintains mitochondrial health is called mitophagy. Previous work from our lab shows that autophagy-dependent cancer cells can acquire resistance to autophagy inhibition and exhibit normal mitochondrial function. This indicates that cells can survive without autophagy and mitochondrial quality control. Previously, our lab showed that autophagy-deficient cells upregulate an autophagy-independent form of mitochondrial degradation known as Mitochondrial-Derived Vesicles (MDVs). These tiny vesicles bud off the mitochondria and directly traffic to the lysosome, bypassing autophagosomes. However, upstream regulators of MDVs remain largely unknown. To understand how autophagy-deficient cells maintain their mitochondrial health without autophagosomes, we performed a genome-wide CRISPR screen using mitochondrial-localized pH-sensitive probes to identify novel regulators of mitochondrial delivery to the lysosome. This screen uses mCherry-GFP-Fis1 which localizes to the outer membrane of mitochondria and we have shown that ratiometric flow cytometry can quantify the amount of mitochondria that are in an acidic environment like a lysosome. One of the top hits from this screen was VPS35, a core component of the retromer complex that is involved in protein trafficking from endosomes to the Golgi. We are currently elucidating the mechanisms by which VPS35 regulates mitochondrial quality control and testing if VPS35 directly regulates MDV formation or trafficking. These mechanisms could be important in cancer, aging, and neurodegenerative disease which have dysregulated mitochondrial homeostasis pathways.

Investigating Adaptive Metabolic Responses to RAS Inhibition in Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDA) is the fourth-leading cause of cancer-related deaths among adults in the United States. KRAS is mutated in >90% of all PDA cases and has remained an elusive therapeutic target until recently. The KRASG12D inhibitor MRTX1133 and the RAS(ON) inhibitor RMC-6236 have been investigated in preclinical models and were found to potently perturb tumor growth. Despite these groundbreaking advances, resistance to RAS inhibitor treatment occurs rapidly, presenting a significant obstacle to improving patient outcomes. To better understand these resistance mechanisms, we investigated changes in lipid metabolism in response to RAS inhibitor treatment. To investigate lipid metabolic responses to RAS inhibition in PDA cells, we applied 13C stable isotope tracing to PDA models in 2D culture and ex vivo tumor slices. In data from 2D culture experiments, we observed that MRTX1133-resistant PDA cells exhibit lower basal levels of fatty acid synthesis compared to matched parental cells. Moreover, the resistant cells did not exhibit decreases in synthesis when treated with MRTX1133, which was observed in parental cells. In mouse tumor slices treated with RMC-6236, fatty acid elongation was inhibited relative to vehicle-treated slices. Together, these results indicate that RAS inhibition alters fatty acid metabolism in 2D culture and tumor slice models. Findings from these experiments will be used to exploit vulnerabilities in tumor metabolism and inform treatment strategies to ultimately improve patient outcomes.

Exploring Treg Gene Regulation by Screening and Characterizing Treg-specific cis-Regulatory Elements

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Regulatory T cells (Tregs) are essential for immune homeostasis, and their dysfunction can trigger autoimmune diseases. Cis-regulatory elements (CREs) play a critical role in regulating target gene expression, with different CREs influencing various stages of cellular development, responding to distinct pathways, and shifting during cell differentiation. We propose that a deeper understanding and characterization of CREs that control Treg development and function could provide valuable insights into currently unknown aspects of Tregs, such as the functional differences between Treg subsets and their behavior in different microenvironments. Our research focuses on identifying previously unidentified CREs that regulate Treg function. We aim to identify novel CREs controlling Foxp3 expression using CRISPR tiling screens and validate their function both *in vitro* and *in vivo*. Additionally, we will map Treg-specific enhancer-gene connections using a CRISPRi/dCas9 sgRNA library to explore the roles of enhancers in Treg populations. Our study aims to provide insights into potential treatments for diseases caused by mutations in these elements and to modulate Treg function in specific microenvironments, such as inflammation and tumors, preventing systemic adverse effects and offering more precise therapeutic strategies.

PolyIC-Loaded Ionizable Lipid Nanoparticles Sustain Hepatocellular Interferon Signaling to Enhance CD8+ T Cell-Mediated Anti-Tumor Immunity in Liver Cancer Therapy

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Liver cancer is a leading cause of cancer-related deaths worldwide, with a five-year survival rate of less than 20%. There still remains a significant unmet need for liver cancer patients. Previous studies, including our own, have shown that polyIC holds promise in cancer prevention; however, its clinical application is limited by high toxicity. In this study, we aimed to enhance the therapeutic efficacy for liver cancer by engineering lipid-based nanoparticles to deliver polyIC specifically to the liver. polyIC-loaded lipid-based nanoparticles (polyIC-LNPs) demonstrated potent tumor-suppressive effects in both primary and metastatic liver tumor models, accompanied by a manageable toxicity profile. Notably, even a single dose of polyIC-LNPs was sufficient to significantly inhibit tumor growth. Single-cell RNA sequencing (scRNA-seq) analysis revealed that polyIC-LNP treatment promoted a proinflammatory tumor microenvironment and enhanced the CD8+ T cell-mediated antitumor immune response. Mechanistically, polyIC-LNPs induced robust and sustained production of type I interferons and upregulated the expression of downstream interferon-stimulated genes (*Cxcl9*, *Cxcl10*, and *Ccl2*). This promoted the interaction between CD8+ T cells and conventional dendritic cells (cDC1) in the liver, ultimately enhancing CD8+ T cell proliferation and activation. Depletion of CD8+ T cells or cDC1 significantly abrogated the antitumor effects of polyIC-LNP treatment. Our findings highlight the potential of polyIC-LNPs as a novel therapeutic approach for liver cancer, effectively stimulating hepatic interferon signaling and enhancing liver immunity to combat liver cancer.

Nutrient Driven Histone Code Determines Exhausted CD8 T Cell Fates

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Exhausted T cells (TEX) in cancer and chronic viral infections undergo metabolic and epigenetic remodeling, impairing their protective capabilities. However, the impact of nutrient metabolism on epigenetic modifications that control TEX differentiation remains unclear. We showed that TEX cells shift from acetate to citrate metabolism by downregulating acetyl-CoA synthetase 2 (ACSS2) while maintaining ATP-citrate lyase (ACLY) activity. This metabolic switch increased citrate-dependent histone acetylation, mediated by histone acetyltransferase KAT2A-ACLY interactions, at TEX signature-genes while reducing acetate-dependent histone acetylation, dependent on p300-ACSS2 complexes, at effector and memory T cell genes. Nuclear ACSS2 overexpression or ACLY inhibition prevented TEX differentiation and enhanced tumor-specific T cell responses. These findings unveiled a nutrient-instructed histone code governing $CD8^+$ T cell differentiation, with implications for metabolic- and epigenetic-based T cell therapies.

Degradation of PKC Theta is a Checkpoint in the T Cell Response to Cancer

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Antigen signaling is fundamental to the T cell response to cancer. Chronic antigen signaling drives dysfunction, or exhaustion, in anti-tumor CD8 T cells, but how antigen controls differentiation in exhaustion is unknown. We found that two protein kinase C (PKC) paralogs, PKC-θ (theta) and PKC-η (eta), have opposing functions downstream of the T cell receptor. PKC-θ is necessary to sustain the T cell response, but antigen signaling induces its degradation. We engineered a degradation-resistant variant of PKC-θ, and its introduction to CD8 T cells improved their response to cancer and chronic viral infection. PKC-θ and -η drive distinct phosphoproteomes, and PKC-η operates independently of the canonical PKC-θ signaling complex. Elimination of detrimental aspects of PKC-η signaling by ablating its putative downstream target, CK1G2, improved the anti-tumor response in mice and human T cells. Our study shows how T cells can employ parallel, differentiation-dependent pathways to interpret an antigen signal and how exploiting these subset-specific signaling pathways can lead to new immunotherapies.

Epigenetic Control of Tumor Associated Macrophages – Targeting the BAF Nucleosome Remodeling Complex

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The BAF complex is a multi-subunit nucleosome-remodeling complex. Inhibitors of the BAF complex have entered phase I clinical trials due to the dependency of several cancer types on the BAF complex. We hypothesized that BAF complex inhibitors would impact not only tumor cells directly, but also affect cells of the tumor microenvironment.

Subcutaneous growth of MC38 adenocarcinoma was substantially slowed in mice treated with BAF inhibitors (FHD-286 or BRM014) and anti-PD-L1 compared to mice treated with PD-L1 alone. Multiome scRNA and scATAC sequencing revelated changes in the gene expression and chromatin accessibility across several cell types, including upregulation of interferon signature genes (ISGs) in tumor cells, CD8+ T cells and macrophages. Tumor associated macrophages (TAMs) displayed reduced expression of CD206, associated with immunosuppression, and increased expression of antigen presentation (MHC-I) and co-stimulation (CD86) proteins, identifying a previously unrecognized role of the BAF complex in TAM function.

To directly study the role of BAF in TAMs, we used a mouse model with myeloid-specific (LysM-Cre and Itgax-Cre) deletion of ARID1A (the largest subunit of the canonical BAF complex). Growth of tumor lines was slowed in mice lacking myeloid-ARID1A compared to controls and the response to anti-PD-L1 was boosted, phenocopying effects observed with BAF-complex inhibitors. ARID1A-deleted TAMs displayed widespread changes in chromatin accessibility and gene expression, including an enrichment of ISGs. Upregulated ISGs with increased promoter accessibility included the gene-encoding the co-stimulatory CD86 molecule, known to be important for activation of T cells by antigen-presenting cells. *In vitro* experiments established that the upregulation of Cd86 in ARID1A-deficient macrophages was not dependent on soluble factors, but could be hyperinduced by interferon or co-culture with T cells. *In vivo*, cells lacking myeloid-specific ARID1A showed increased CD8+ T cell activation. Using depleting and blocking antibodies respectively, we established the tumor growth phenotype was dependent on CD8+ T cells, and partly dependent on CD86, highlighting the role of TAM:CD8+ T cell cross talk in promoting an anti-tumor immune response.

Collectively, we discovered the BAF complex is a regulator of tumor-associated macrophages and identified a molecular and cellular mechanism through which myeloid-specific ARID1A-loss boosts anti-tumor immunity.

Understanding the Role of NUAK1 in Mucinous Appendix Cancer

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Mucinous Appendiceal Cancer (MAC) is a rare aggressive form of cancer, with poor patient survivability outcomes. ATAC Seq data shows increased chromatin exposure at the NUAK1 promoter region in MAC compared to normal appendix leading us to hypothesize that increased NUAK1 contributes to the cancer phenotype. Mechanistically, NUAK1 activates an antioxidant response pathway, which ultimately inhibits cancer cells from undergoing ferroptosis. We hypothesize that inhibiting NUAK1 expression pathway will make cancer cells more susceptible to ferroptosis, less cell migration, survival and proliferation.

We will silence NUAK1 expression by genetic knockdown and pharmacological inhibition using selective NUAK1 inhibitor, HTH-01-015, to understand NUAK1's role in cell survival, proliferation, and migration. EdU will be used to visualize and quantify proliferating cells and a scratch assay will be used to track cell migration. In addition, ferroptosis inducers, RSL3 or Erastin, will be used with NUAK1 inhibition to see if cells are more susceptible to ferroptosis in the presence of NUAK1 silencing and inhibition. Cleave caspase staining will be used to measure apoptosis. Due to limited appendiceal cancer cells, we are running our experiments on human Pancreatic Ductal Adenocarcinoma (PDAC) line, Panc1, and Patient Derived Organoids (PDOs) hf3, hf44, and hM1A for proof of concept.

Data from scratch assays show that knocking down NUAK1 expression decreases the rate of cell migration and proliferation in PDAC cell lines. The rate of cell proliferation to close the scratch in the scratch assay was slower in NUAK1 silenced cells compared to control cells ($p<0.05$). NUAK knockdown was validated via rt-qPCR.

Furthermore, inhibiting NUAK1 expression, via HTH-01-015, in tandem with inducing ferroptosis, via RSL3, leads to a greater amount of apoptosis as compared to the control group ($p<0.05$), NUAK1 inhibition alone ($p<0.05$), or oxidative stress conditions alone ($p<0.05$).

Organoids treated with HTH-01-015 alone have statistically fewer cells undergoing cell proliferation compared to control ($p=0.0039$), whereas organoids treated with a combination of HTH-01-015 and Erastin have even fewer proliferating cells compared to control ($p<0.0001$).

The data collected thus far demonstrates the role NUAK1 plays in increased rate of proliferation, migration, cell survival, and resistance against oxidative stressors. Further exploring NUAK1 inhibition as a potential treatment for patients with MAC is promising, as NUAK1 inhibition sensitizes cancer cells to ferroptosis, and can ultimately make patients more responsive to treatments.

Sensitizing cells to ferropsosis via NUAK1 inhibition.

Utilizing Oxidative Mitohormesis to Combat T Cell Exhaustion

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Programmed alterations in mitochondrial metabolism, dynamics, and biogenesis drive T cell fate trajectories. For instance, mitochondrial dysfunction has been closely linked with T cell exhaustion, particularly through alterations in biogenesis and enhanced oxidative stress through the production of reactive oxygen species (ROS). The concept of adaptive preconditioning to acute stress, or mitohormesis, has been shown to confer a number of beneficial physiological effects and promote longevity. Our lab has generated a unique mouse model of mitochondrial oxidative mitohormesis that is based on an inducible and reversible knockdown of the mitochondrial superoxide dismutase to transiently increase mitochondrial superoxide stress (iSOD2 mice). We previously showed that mice that undergo this form of mitochondrial stress only during embryogenesis exhibited a mitohormetic response in the adult liver that was characterized by enhanced mitochondrial mass and antioxidant capacity driven by NRF2 and PGC-1α. Similarly, iSOD2 mouse embryonic fibroblasts (MEFs) that underwent transient SOD2 knockdown and recovery *in vitro* also showed enhanced resistance to subsequent menadione- induced oxidative stress. We wanted to employ this model to investigate if oxidative mitohormesis could also help to alleviate T cell exhaustion. To do this, we crossed the iSOD2 mice to P14 CD8+ TCR transgenic mice and confirmed that *in vivo* administration of doxycycline led to successful knockdown of SOD2 and heightened superoxide production in CD8+ T cells. Next, we sorted and adoptively co-transferred both control and iSOD2 CD8+ T cells into recipient mice and allowed them to recover for one week before infecting them with chronic LCMV clone 13 virus. While there was no significant alteration in overall cell number, the iSOD2 CD8+ T cells showed a slight, but significant reduction in the proliferation marker Ki67+ at day 7 post-infection in the spleen. Interestingly, we found reduced reactive oxygen species markers (both mitochondrial and cellular) in the iSOD2 CD8+ T cells compared to controls in the spleen at days 7 and 21-post infection. This also correlated with a significant reduction in progenitor and terminal exhaustion markers, as well as enhanced granzyme B production in the iSOD2 CD8+ T cells. These findings suggest that oxidative mitohormetic reprogramming may help mitigate T cell exhaustion. We plan to further characterize these iSOD2 CD8+ T cells to determine global transcriptional, metabolic and epigenetic changes and extend our findings to cancer models as well. These studies indicate that mitohormetic interventions may provide favorable outcomes in T cells to help improve tumor immune response and immunotherapy.

CRISPR Saturation Screens Help Unveil Hotspots of Resistance to SHP2 Inhibition

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The phosphatase SHP2 plays a central role in signaling pathways activated by receptor tyrosine kinases (RTKs), playing a crucial role in the regulation of cell growth and differentiation. In many human cancers, including acute myeloid leukemia (AML), SHP2 hyperactivation through mutations or increased activity leads to uncontrolled cellular proliferation and survival. Several allosteric SHP2 inhibitors are currently being tested in clinical trials. Since most targeted anti-cancer therapies eventually fail due to mutations in the target protein or through the genetic or epigenetic modulation of compensatory pathways, we sought to comprehensively characterize critical hotspots in SHP2 that help engender resistance to allosteric SHP2 inhibition. We reasoned that this approach may help anticipate mutations that may emerge following SHP2-targeted therapies. For this, we used CRISPR saturation mutagenesis screening, which can help achieve single amino-acid residue level resolution of target mutant selection. Using custom pooled oligo synthesis, we generated an sgRNA library of ~1,000 sgRNAs tiling the genomic region spanning all coding exons of SHP2. Using this stably expressed lentiviral library in Cas9-expressing MOLM13 human AML cells, we conducted CRISPR-based fitness screens. Analysis of the data revealed several sgRNAs with >1,000-fold enrichment in MOLM13 cells treated with SHP099 compared to DMSO-treated cells. Interestingly, these highly enriched sgRNAs clustered around two main regions – one was the N-terminal region (aa Q57-E76) spanning the SH2 domain and the other - the C-terminal phosphatase domain (aa R498-E508). Interestingly, these two regions are normally involved in facilitating the interaction between two domains which keep SHP2 in an autoinhibited configuration. Thus, enrichment of mutations in these two sites suggests that an aberrantly activated SHP2 will escape inhibition from SHP099 and related allosteric inhibitors. In addition to this, our studies also helped identify sgRNAs enriched in SHP099 binding sites in SHP2 (aa E128-H132 and V490-M496), demonstrating the power of this technology in identifying drug-target interfaces, and in generating drug-escape mutants. Sequencing of SHP2 from individually selected single cell clones revealed the exact amino acid substitutions mediating resistance, including mutations frequently observed in treatment-naïve human cancer. Taken together, our studies reveal the power of the CRISPR saturation mutagenesis approach to identify and anticipate mutations that may emerge following targeted therapies in an experimental setting. In the clinical setting, since SHP2 inhibitors are being evaluated in combination with other therapies, our studies suggest that monitoring for emergent SHP2 mutations in the N-terminal SH2 domain or the PTP domain might be warranted.

Identifying and Characterizing Novel Myc Regulated Microproteins

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The C-Myc (MYC) oncogene is over-expressed in >50% of cancers. The lack of binding pockets and nuclear localization of MYC has deemed it "undruggable", leading to extensive exploration of its downstream targets. However, emerging research highlighting the pervasive translation of noncanonical open reading frames (ORFs) has revealed that our annotation of proteins is severely incomplete. These novel proteins (microproteins) are encoded by short ORFs (sORFs), whose presence and function in relation to MYC's wide arsenal are not known. This is due to many limitations, such as the small size of these genomic regions, rendering them difficult to measure experimentally and align computationally. Recent advances have lowered this barrier. Here, we introduce a computational framework integrating advances across different fields of biology (evolutionary conservation, ribosome profiling, patient GWAS data, and existing datasets) designed to find novel genomic regions with high likelihood of being protein coding elements with functional significance. We address this gap in knowledge in B-cell lymphoma, a paradigm for MYC-driven cancer. We integrated RNA-seq and ChIP-seq data of P493-6 human-derived B-cell lymphoma cell lines with a doxycycline-inducible "Tet-off system" for MYC expression to find both coding and noncoding RNA upregulated by and bound by MYC. We then developed a bioinformatics pipeline to find microproteins downstream of MYC activity, located in UTR regions or lncRNA. This approach results in a new set of proteins integral to MYC-driven oncogenesis in tissue-specific settings and possibly other cancers, and has the potential to be applied to different biological systems.

Lactate-responsive NDRG3 is an Essential Regulator of T Cell Expansion and Function

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One of the most common alterations in the tumor microenvironment is an increased presence of lactate, which, for years, has been thought to be immunosuppressive. Studies have shown that culturing T cells in the presence of lactic acid suppresses effector cytokine production. However, recent work has challenged the idea that lactate is truly immunosuppressive as culturing T cells with sodium lactate does not compromise cytokine production, it actually increases IFN_ expression. Additionally, the function of Lactate Dehydrogenase A (LDHA), which produces lactate, was found to be necessary for optimal effector T cell expansion and cytokine production. This result is particularly interesting because it is commonly thought that the main role of LDHA is to recycle NAD, but it apparently has broader effects on T cell differentiation and function, indicating that the enzymatic product, lactate, could serve a purpose as well. In fact, we are uncovering a novel role for lactate in T cells as a signaling molecule that helps promote critical T cell effector programs. We have found that the lactate-responsive protein N-myc Downstream Regulated Gene 3 (NDRG3) is critical for *in vivo* T cell expansion and functional effector cell differentiation. Lactate helps prevent the degradation of NDRG3 by associating with NDRG3 and hindering the binding of the VHL complex. Conceptually, this work suggests that lactate acts as a 'metabolic checkpoint' for T cells, where lactate levels may be interpreted by the T cell (through NDRG3 levels) as an indicator of a permissive metabolic state to fuel proliferation and survival.

Perturbing the Function of the OSM Receptor in the Pancreatic Ductal Adenocarcinoma (PDA) Tumor Microenvironment (TME)

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Pancreatic ductal adenocarcinoma (PDA) is a highly lethal cancer with a low survival rate and significant global mortality impact, having the lowest 5-year relative survival rate for all stages combined in the United States at 13%. In 2024, an estimated 66,440 new PDA cases and 51,750 deaths are expected. The interaction between the immunosuppressive tumor microenvironment (TME) in PDA, characterized by limited CD8 T cell infiltration and an imbalance of cytokines, underscores the need to explore mechanisms underlying the poor immune responses in order to improve immunotherapies and adjuvant treatments. Oncostatin M (OSM), an interleukin-6 (IL-6) family cytokine, and its receptor (OSMR) have been linked to poor overall survival in pancreatic and other cancers, yet its role in PDA remains poorly understood. Based on our previous discovery that LIF, another IL-6 family member, plays a driving role in PDA acting on tumor cells, our objective is to conduct preclinical studies to ascertain the possibility of targeting the OSM-OSMR axis in PDA. Our study will examine the role of OSM/OSMR signaling in PDA development, focusing on its capacity to regulate TME and trigger inflammatory responses. We aim to define the significance of the OSM-OSMR axis in PDA initiation and progression, and uncover potential therapeutic targets, including OSM itself, that may ultimately lead to new targeted therapies for this aggressive cancer.

Defining Metabolic Zones from Spatial Transcriptomics with MetaZone in Pancreatic Tumor Microenvironment

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The distinct metabolic preferences of various cell types within the tumor microenvironment (TME), along with vasculature, create different metabolic niches. As tumors evolve, they compete with stromal cells and immune cells for key nutrients, influencing the metabolic state of immune cells, which in turn alters their infiltration, functions, and survival. In this context, studying the spatial distribution of immune cells within metabolic niches is crucial. To this end, I develop a bioinformatics algorithm MetaZone on the Xenium spatial transcriptomics (ST) datasets to decipher the metabolic zones within the TME of pancreatic ductal adenocarcinoma (PDAC).

A Chemical Biology Approach to Identify Small Molecule Modulators of γδ T Cell Activation

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γδ T cells exhibit both innate and adaptive-like properties of the immune system and play key roles in cancer immunity and infection. Vγ9Vδ2 T cells, the majority γδ T cell subset in peripheral blood, are activated by phosphoantigen (pAg) metabolites to exert cytotoxic functions against malignant cells. Previous discoveries determined that butyrophilin 3A1 (BTN3A1) uniquely binds to pAgs that act as molecular glues to form the BTN2/3A1 complex, where BTN2A1 directly binds the Vγ9Vδ2 TCR and subsequently induces Vγ9Vδ2 T cell activation. However, the downstream mechanisms that induce γδ T cell-mediated antitumor activity have yet to be fully elucidated. Here, we developed a high-throughput cell-based co-culture screening assay of ~50,000 small molecule compounds using an integrated flow cytometry and bioinformatics approach. We identified select hit compounds that induce CD69 surface expression and IFNG mRNA expression of Vγ9Vδ2 TCR+ Jurkat cells when co-cultured with target cancer cells and suboptimal doses of Zoledronic acid, a known inducer of Vγ9Vδ2 T cell activation. This project aims to further elucidate the mechanisms in which BTN2A1 and BTN3A1 induce antitumor functions of Vγ9Vδ2 T cells and potential avenues to utilize Vγ9Vδ2 T cells in cancer immunotherapeutic strategies.

Using Ex Vivo Tissue Slice Culture to Elucidate Stromal Contributions in PDAC Tumor Metabolism

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Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy for which effective therapeutics are limited. The development of therapeutics is plagued by poor translation of preclinical success into clinical settings as well as a high rate of acquired resistance rates. Metabolic dysregulation within these tumors can confer both survival and resistance. This metabolic dysregulation extends beyond tumor cells themselves and involves contributions from the notably diverse PDAC tumor microenvironment (TME). However, methods for investigating these stromal contributions to tumor metabolism are limited. *In vitro* tissue culture models fail to account for the native cellular diversity and distribution within the tumor, while *in vivo* methods do not allow for the separation of these local contributions from systemic physiology. To investigate the role of the TME in PDAC tumor metabolism, we developed a methodology for performing metabolic flux assays in ex vivo cultures of organotypic PDAC tumor slices using stable isotope tracers. We quantified metabolites across multiple pathways using GC-MS and LC-MS/MS methods and determined key biosynthetic fluxes within these tumors. Tumor material from multiple models of PDAC was analyzed, including a genetically engineered mouse model (KPC), orthotopic tumors and xenografts, and a surgically resected human PDAC tumor. By quantifying key metabolic fluxes within each model and comparing it to data obtained with 2D tissue culture methods, we identified that the tumor microenvironment contributes critically to nucleotide and lipid metabolism. By gaining insight into the role of the TME in PDAC metabolism, we can better understand the limitations of *in vitro* and *in vivo* models of PDAC and how those limitations may impact the investigation of potential therapeutics as well as the identification of new potential targets.

Role of Maternal Factors in Determining Offspring Intestinal Immunity and Intestinal Related Diseases

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The influence of maternal factors on the offspring intestinal immune system in early life, while recognized, is mechanistically unclear. Our studies using a cross-fostering setup with B6 mice raised by either B6 or Balb/c dams revealed that high maternal IgA transfer from Balb/c dams reduces microbe-specific regulatory T cells (RORγ+ Tregs) in B6 offspring. We hypothesized that maternal IgA, essential for pioneer microbiota colonization, along with other bioactive factors in breast milk, shapes the intestinal immune microenvironment, influencing T cell differentiation and function, and thereby affecting susceptibility to colitis and colorectal cancer (CRC). Indeed, in a chemically induced chronic colitis model, B6 mice raised by Balb/c dams showed increased susceptibility to colitis compared to mice raised by B6 dams. We then sought to understand the mechanisms by which maternal factors determines intestinal RORγ+ Tregs. Temporal analysis of the intestinal immune microenvironment in mice raised by B6 vs Balb/c dams through single-cell RNA sequencing and flow cytometry revealed striking changes in an enriched population of B cells expressing interferon-stimulated genes (ISGs) in mice raised by Balb/c dams. The changes in B cell populations preceded the appearance of RORγ+ Tregs and we are currently exploring the role of these immune cell changes in determining intestinal RORγ+ Tregs. Overall, our results highlight the significant role of maternal factors in shaping immune cell populations and colitis susceptibility, offering insights into early life immune programming and identifying potential therapeutic targets for intestinal diseases.

Genomic and Transcriptomic Analyses of Chemical Hepatocarcinogenesis Aggravated by Loss of Oncoproteins

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Background and Aims: The chemical carcinogen diethylnitrosamine (DEN) is often used to induce hepatocellular carcinoma (HCC) in mice. Curiously, several labs have reported that removal of oncoproteins from hepatocytes exacerbated DEN-induced HCC, with mechanisms unknown. This study aimed at deciphering molecular mechanisms underlying the tumor suppressive effect of oncoproteins. Methods and Results: We generated mutant mouse lines with hepatocyte-specific deletions of Met, Ptpn11/Shp2, Ikkβ, or ctnnb1/β-catenin, and assessed DEN-induced tumorigenesis in the wild type (WT) and mutant mice. To systematically examine genetic and molecular signaling alternations, we performed whole exome and RNA sequencing on liver samples collected at the pre-cancer and established cancer stages. Although the mutational profiles of DEN-induced tumors were barely different in WT and mutant mice, oncoprotein ablation increased DEN-induced mutational burdens, especially in Shp2-deficient tumors. RNA-sequencing revealed multiple changes in signaling pathways, in particular upregulated epithelial-mesenchymal transition (EMT), cell migration and tumor metastasis as well as downregulated small molecule metabolism that were affected by oncoprotein ablation. We identified key molecules and pathways that are associated with hepatic innate immunity and implicated in liver tumorigenesis. In addition, we unveiled markedly changed expressions of a few miRNAs in human HCC database. Conclusion: The aggravation of DEN-induced HCC progression seen on oncoprotein ablation could be caused by common and distinct genomic and signaling alterations. This study reveals a new level of complexity in hepato-carcinogenesis and elucidates molecular mechanisms underlying tumor evolution and recurrence.

PIN1 Prolyl Isomerase Promotes Initiation and Progression of Bladder Cancer Through the SREBP2-mediated Cholesterol Biosynthesis Pathway

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The identities of the functional pSer/Thr.Pro protein substrates of PIN1 and its effects on downstream signaling in bladder carcinogenesis are unknown. Phenotypically, we found that PIN1 positively regulated cell proliferation, stemness maintenance, cell invasion, migration, and urothelium clearance capacity in human/mouse bladder cancer cells and organoids formed from mouse primary urothelial cells *in vitro*. Moreover, *in vivo* subcutaneous xenograft and allograft transplantation mouse tumor models combined with an orthotopic implantation tumor model were utilized to confirm a positive role for PIN1 in controlling tumor growth and potential metastasis. Mechanistically, we observed a negative enrichment of expression of SREBP2-driven cholesterol metabolism pathway genes in PIN1 knockout bladder cancer cells. Consistent with this, we found that free/total cholesterol levels were lower in PIN1 KO bladder cancer cells and showed that they had a lower proliferation than control cells in delipidated medium, which could be restored by exogenous cholesterol. Focusing on SREBP2, the master transcription factor for the cholesterol biosynthesis pathway, as a possible target for PIN1, we showed that PIN1 interacted via its WW domain with SREBP2 following its phosphorylation by the JNK MAP kinase at Ser455, which lies near the Site-2 cleavage site that generates the active, nuclear form of SREBP2. We propose that cholesterol biosynthesis in bladder cancer cells is promoted by PIN1-mediated isomerization and JNK-mediated phosphorylation of SREBP2. Therapeutically, a combination of the sulfopin covalent PIN1 inhibitor and the simvastatin HMGCoA reductase inhibitor suppressed cell proliferation *in vitro* and tumor growth *in vivo* synergistically. Together, these findings emphasize that PIN1 can act as a driver and potential therapeutic target in bladder cancer.

An Ex-Vivo Organotypic Culture Platform of Mucinous Carcinomatosis Peritonei Identifies CDK4/6 Inhibition as a Novel Treatment

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Concomitant mutations in KRAS and GNAS have been linked to mucinous histology in gastrointestinal neoplasms of the appendix, colon, and pancreas (IPMN). Upon progression, these tumors are characterized by metastasis which favors the peritoneal surface, resulting in mucinous carcinomatosis peritonei (MCP). Recently we have described a novel technique which allows for long term culture and drug intervention of human MCP tumors ex-vivo. With evidence of durable clinical benefit of CDK4/6 inhibition in a single patient with chemo refractory MCP of appendiceal origin, we sought to further investigate anti-tumor responses using CDK4/6 inhibition in ex-vivo tumor slices, in order to identify candidates for a personalized clinical therapy trial. Here, in a comparison of ex-vivo patient tumor slices from 18 individual donors, treatment using CDK4/6 inhibitors revealed a significant reduction in cancer cell specific proliferation in mucinous GNAS-mut tumors compared to non-mucinous cancers. Anti-tumor responses of CDK4/6 were determined to be tumor-cell intrinsic, as CDK4/6 inhibition blocked proliferation of stromal cells in the TME independently of patient tumor mutational and mucin status. Based on these finding a personalized clinical trial was conducted where we report that 13 of 16 patients (81%) enrolled with MCP treated with palbociclib had at least a 10% decrease in CEA, as compared to historical chemotherapy responses rates reported from 14-30%. These results indicate that CDK4/6 inhibition is a novel and efficacious treatment for patients with MCP.

Modeling Compound Lipid Homeostasis Using Stable Isotope Tracing

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Lipids represent the most abundant and diverse pool of metabolites found in cells, facilitating compartmentation, signaling, among other critical functions. Given these integral roles, dysregulation of lipid metabolism is linked to disease states such as cancer and neurodegeneration. However, limited tools are available for quantifying metabolic fluxes across the lipidome beyond fatty acid synthesis or glycerolipid backbone turnover. To directly measure reaction fluxes encompassing compound lipid homeostasis, we applied stable isotope tracing, high-resolution mass spectrometry, and network-based isotopologue modeling to non-small cell lung cancer (NSCLC) models. Compound lipid metabolic flux analysis (CL-MFA) enables the concurrent quantitation of fatty acid synthesis, elongation, desaturation, compound lipid backbone synthesis, headgroup assembly, and salvage reactions within virtually any biological system. Here, we resolve liver kinase B1 (LKB1)-mediated regulation of sphingolipid recycling in NSCLC cells and precision-cut lung slice cultures. We also demonstrate that widely used tissue culture conditions drive cells to upregulate fatty acid synthase flux to supraphysiological levels to maintain compound lipid pools. Finally, we identify previously uncharacterized isozyme specificity of ceramide synthase inhibitors, highlighting the molecular detail revealed by CL-MFA.

Convergent Regulation of the CIITA-MHC II Pathway by PPARδ and Type 1 Interferon in Intratumoral Treg Cells

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Regulatory T cells (Treg cells) play an essential role in suppressing anti-tumor immunity, often resulting in unfavorable clinical outcomes across numerous cancers. However, systemic Treg depletion, while augmenting anti-tumor responses, also triggers detrimental autoimmune disorders. Thus, dissecting the mechanisms by which Treg cells navigate and exert their functions within the tumor microenvironment (TME) is pivotal for devising innovative Treg-centric cancer therapies. Our study highlights the role of peroxisome proliferator-activated receptor β/δ (PPARδ), a nuclear hormone receptor involved in fatty acid metabolism. Remarkably, PPARδ ablation in Treg escalated tumor growth and augmented the immunosuppressive characteristics of the TME. This absence of PPARδ spurred an increased expression of genes central to antigen presentation, notably CIITA and MHC II. Our results showcase a novel association where the absence of CIITA in PPARδ-deficient Treg bolsters anti-tumor responses, casting CIITA as a pivotal downstream regulator of PPARδ within Treg. *In vitro* assays demonstrated that elevated CIITA levels enhance the suppressive capacity of Treg, facilitated by an antigen-independent interaction between Treg-MHC II and Tconv-TCR/CD4/Lag3. A significant revelation was the role of type 1 interferon as a TME signal that promotes the genesis of MHC II+ Treg; PPARδ deficiency intensifies this phenomenon by amplifying type 1 interferon signaling, mediated by a notable upsurge in JAK3 transcription and an increase of pSTAT1-Y701. In conclusion, the co-regulation between TME cues and PPARδ signaling shapes the adaptive and suppressive roles of Treg cells through the CIITA-MHC II pathway. Strategically targeting the potent MHC II+ Treg population could open a new avenue for cancer therapies by boosting anti-tumor defenses while curbing autoimmune threats.

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