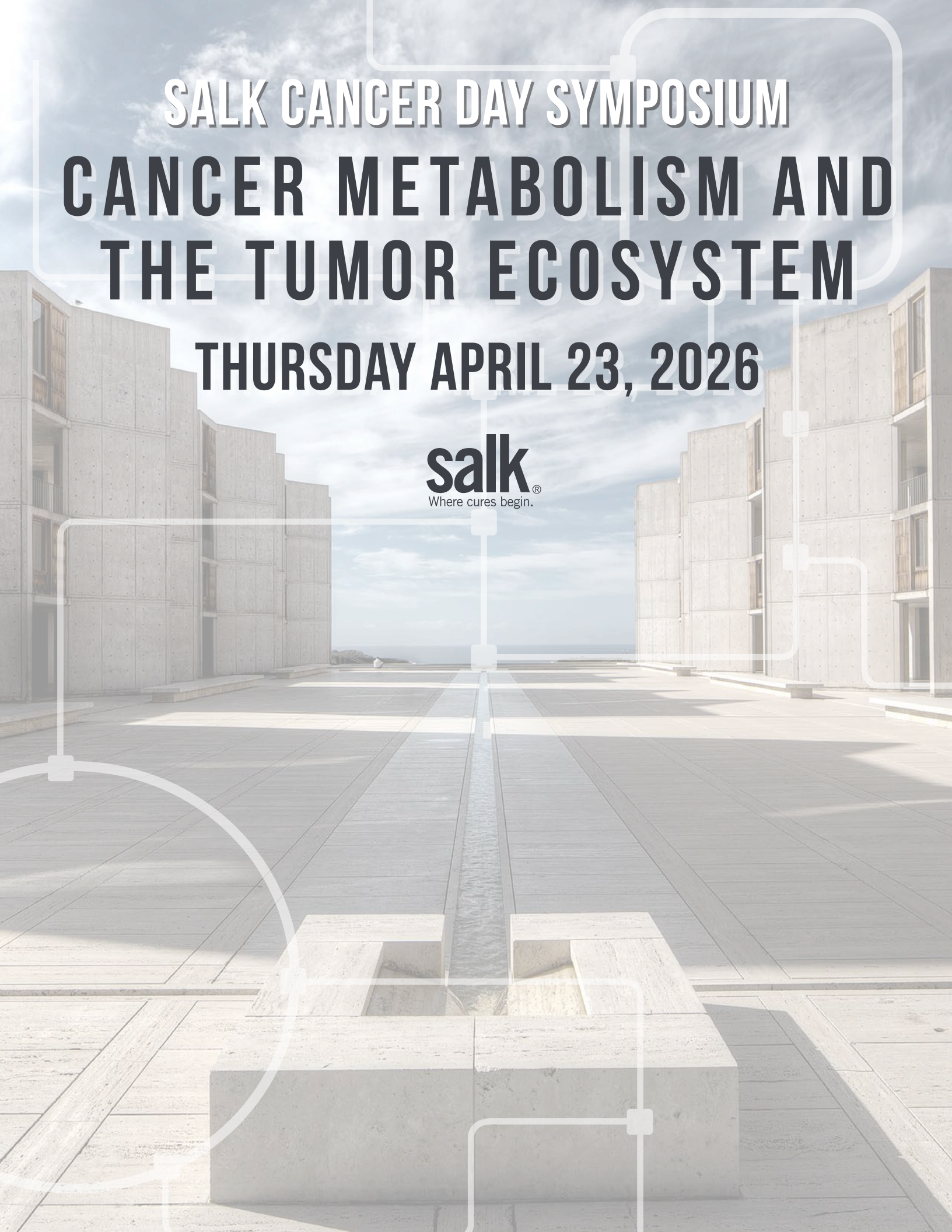

SALK CANCER DAY SYMPOSIUM

CANCER METABOLISM AND THE TUMOR ECOSYSTEM

THURSDAY APRIL 23, 2026

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Abstracts of papers presented at

Salk Cancer Day Symposium

April 23, 2026

Organized by Salk Institute Researchers:

Samuel Bloom, PhD
Casie Kubota, PhD
Joel Encarnacíon-Rosado, PhD
Victoria Osorio Vasquez, PhD
Taylor Morgan, Lab Admin Manager

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Dissecting the epigenetic inter- and intra-tumor heterogeneity of glioma at single-cell resolution

POSTER ABSTRACTS

Fluorescence imaging of actin in MCF7-shMLH1 cells: Unraveling actins role in breast cancer progression

Monica Ambrocio[1], Megha Raghunathan[2], and Carlos Luna Lopez[1]
[1]Department of Biological Sciences, California State University San Marcos, San Marcos, CA 92096
[2]Department of Biology, San Diego State University, San Diego, CA 92182

In the United States, one out of every eight women will be diagnosed with breast cancer during their lifetime. A key factor in what makes cancer cells dangerous is their ability to metastasis and travel throughout the body. The progression of metastatic cells is formed at subcellular levels. Proteins such as actin and vinculin are vital to cytoskeletal motility and overall cellular structure. The rearrangement of actin proteins and fibers is a critical key in understanding cell metastases.

Our objective is to investigate the impact of MLH1 Knockdown on actin cytoskeleton formation in breast cancer cells, with the focus on stress fiber formation and its potential implications for metastatic behaviors. MLH1 is a key DNA protein for mismatch repair, and its deficiency and mutation has been linked as major drivers of cancer. Defects in the MutL complex of mismatch repair, comprised of MLH1 and PMS2, were recently identified as drivers of endocrine treatment resistance in 15–17% of ER+/HER2- breast cancer. MLH1 also exhibits relationships with several proteins involved in cytoskeletal organization, but the role of MLH1 and actin is still under investigation. To assess the effects of MLH1 Knockdown, following incubation, fluorescence imaging using Phalloidin and Vinculin was performed on MCF7-shMLH1, mutants(P747I, L471S) and the control, shLUC, to visualize changes in the structure, distribution of actin and vinculin formation.

Fluorescence imaging analysis reveals actin intensity in shMLH1 is more prevalent at the edges of each cell. Furthermore, there was a lack of stress fiber formation, which suggests that MLH1 influences critical cellular processes that alter actin dynamics. The formation of Vinculin is still under investigation. The lack of stress fiber formation could lead to weakened cell-matrix interactions, thereby facilitating cell detachment and enhanced motility, both crucial steps in cancer metastasis. By analyzing how the MLH1 knockdown alters cytoskeletal organization in shMLH1 and shLUC, we can better define how structural changes in cells contribute to metastatic potential. To further our work, we want to extend these findings into more physiologically relevant systems such as incorporating 3D spheroid models. The use of spheroid invasion assays to measure cell migration and growth phenotype coupled with imaging of cytoskeletal dynamics could reveal pathways that link genomic traits to mechanobiology in cancer progression.

Obesogen co-exposure promotes triple-negative breast cancer growth via adipocyte-mediated mechanisms

Sarah M. Bernhardt[1], Mikella Robinson[1], and Carrie D. House[1,2]
[1]Department of Biology, San Diego State University, San Diego, CA, 92182, United States
[2]Moores Cancer Center, University of California, San Diego, La Jolla, CA, 92037, United States

Bisphenol A (BPA) and DDE (the primary metabolite of DDT) are environmental 'obesogens' independently associated with altered adipocyte function and increased breast cancer risk. While the effects of obesogens alone are well studied, particularly in hormone receptor (HR)-positive breast cancer, the biological consequences of combined exposure and their impact on triple negative breast cancer (TNBC) through adipocyte-tumor interactions remains poorly understood. Here, we investigate how obesogen co-exposure alters mammary adipocyte function and promotes tumorigenesis in a mouse model of TNBC.

Post-pubertal BALB/c mice (n=10/grp) were exposed to obesogens via drinking water for the duration of the study, at environmentally relevant doses (Vehicle (ethanol); BPA (4μg/kg/day); DDE (0.8μg/kg/day); combination (BPA+DDE)). After 2 weeks exposure, mammary cancer cells (4T1; 20,000 cells) were injected into the fourth inguinal mammary fat-pads, and investigator-blinded tumor growth tracked for 21 days. To assess how obesogens affect adipocyte function *in vitro*, 3T3-L1 preadipocytes were differentiated in the presence of vehicle (ethanol), BPA (1nM), DDE (1μM), or combination (BPA+DDE), and adipokine secretion profiles assessed.

In tumor-free mammary glands, obesogen exposure associated with increased adipocyte size (BPA 1029±231μm2, DDE 957±350μm2, Combo 1011±344μm2) compared to vehicle-treated mice (766±187μm2; $p<0.05$). Interestingly, while individual exposures induced adipocyte hypertrophy, only combined exposure increased epithelial cell proliferation (Ki67), compared to vehicle-treated mice (2.8-fold, $p=0.02$). In tumors, combination treatment associated with increased tumor burden (1.4-fold, $p=0.02$) and proliferation (1.3-fold, $p=0.04$), compared to vehicle-treated mice. *In vitro*, obesogen exposure altered adipocyte secretion of 11/38 adipokines (>2-fold change), 6 of which were unique to combination-treated adipocytes (up: LIF; down: IGF-II, VEGF, M-CSF, CCL2, adiponectin). Exposure of 4T1 cells to this conditioned media increased proliferation (EdU), compared to vehicle controls (1.5-fold, $p=0.04$).

Co-exposure to BPA and DDE synergistically promotes TNBC proliferation at environmentally relevant doses, underscoring the need to study obesogens in the context of real-world co-exposures. The effects on HR-negative disease were mediated, at least in part, through adipocyte-derived mechanisms, highlighting the need to expand research beyond HR-positive breast cancer.

Predicting T-cell movement from a static microscope image

Jonah Castiglione[1,3], Susanne Alexandra Gamas Vis[2], Leichu Liang[2], Zbigniew Mikulski[2], Miguel Reina-Campos[2,5], and Pallav Kosuri[1,4]
[1]Salk Institute for Biological Studies, La Jolla, CA 92037
[2]La Jolla Institute for Immunology, La Jolla, CA 92037
[3]Department of Computer Science & Engineering, Jacobs School of Engineering, University of California San Diego, La Jolla, CA 92093
[4]Department of Molecular Biology, School of Biological Sciences, University of California San Diego, La Jolla, CA 92093
[5]Department of Pharmacology, School of Medicine, University of California San Diego, La Jolla, CA 92093

Mobile cells are a critical component of the body's defense against cancer. T-cells are frontline soldiers, acting as a surveillance system that recognizes foreign antigens on cancer cells and initiates a direct killing mechanism. The motion of T-cells during these processes contains information regarding their behavioral intentions, a reflection of environmental guidance cues and other chemical information provided by the microenvironment. Analysis of T-cells' motion in tissue therefore has attractive applications, such as identifying sources of chemoattraction, determining T-cell activity, and identifying T-cell subtype/state-specific behaviors. While T-cell dynamics can be measured using *in vivo* intravital microscopy, such measurements are for practical reasons often not possible. Here, we demonstrate that T-cell dynamics can be inferred from static samples through analysis of cell shape. To do this, we introduce PRIZSM (PRediction from Imaging of Zero-Shot Motion), a generalizable model for prediction of T-cell motion from cell morphology. We envision that PRIZSM will enable improved assessment of immune cell activity in fixed tissue biopsies, with applications in basic research and clinical diagnostics.

Rapid optogenetic manipulation of autophagy induces p body formation which include cytoplasmic nuclear pore complexes

Amanda Cyril[1,2], Payel Mondal[1], Louis Parham[1], Dana Mamriev[3], Igor Wierzbicki[4], Celina Shen[1,2], David J Gonzalez[4], Maximiliano A D'Angelo[2], and Christina G Towers[1,2]

[1]Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA

[2]Division of Biological Sciences, University of California San Diego, La Jolla, CA 92037, USA

[3]Cancer Metabolism and Microenvironment Program, NCI-Designated Cancer Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA

[4]Department of Pharmacology, School of Medicine, and the Skaggs School of Pharmacy, University of California San Diego, La Jolla, CA 92037, USA

Autophagy is a highly conserved cellular recycling process that enables degradation and quality control, supporting cellular maintenance. Perturbation of autophagy leads to various cellular responses, such as damaged organelle build-up, depleted nutrient stores, and multiple stress responses. However, the kinetics of these changes has been difficult to study. Current methods of autophagy inhibition provide insight into long-term adaptations but leave the immediate consequences of autophagy inhibition understudied. To determine the rapid effects of autophagy inhibition, we developed the first optogenetic tool to rapidly inhibit autophagy, termed Autophagic Snare for Abrupt Photomanipulation (ASAP). Our approach selectively inhibits autophagy within 5 minutes of blue light, providing a precise and dynamic approach to study autophagy regulation. ASAP is also reversible, and autophagy is reinstated within 2 hours after the removal of light. Proteomic profiling with ASAP revealed the most tightly regulated autophagy substrates along with novel, previously unidentified substrates, including nuclear pore complex (NPC) proteins. Several proteins related to protein condensates, which are often associated with repression of protein translation were also increased. Numerous ribosome binding proteins were decreased immediately after autophagy inhibition, suggesting altered protein translation. We also found that immediately after autophagy inhibition, processing bodies (p-bodies), which can store and degrade translationally repressed mRNAs, increase. Interestingly, other liquid-liquid phase separations like stress granules were not changed with rapid autophagy inhibition. Additionally, we found that induced p-bodies colocalize with accumulating nuclear pore complex proteins including NUP358. A recent report showed that NUP358 is important for p body formation. Therefore, ongoing work is testing our proposed model which states that autophagy inhibition causes an immediate increase in NUP358 leading to p-body formation and repressed protein translation. Future directions include optimization of ASAP for *in vivo* use in model organisms including mice to study the roles of autophagy across physiology and disease, particularly in tumors.

EphA2 signaling in matrix stiffness-regulated breast cancer metastasis

Khoa Doan, Laurent Fattet, Mike W. Matsumoto, Elena Pasquale, and Jing Yang
[1]Department of Pharmacology, University of California, San Diego, School of Medicine
[2]Cancer Center, Sanford Burnham Prebys Medical Discovery Institute

In breast cancer, increased tissue stiffness is one of the key modifications of ECM that is also found in patients with dense breast tissues compared to those with little or no density. Previous studies have shown that higher stiffness promotes epithelial-mesenchymal transition (EMT) and cancer progression. Specifically, EphA2 receptor tyrosine kinase is found to play a critical role in stiffness-induced EMT and invasion. Since EphA2 has also been known to possess complex mechanisms in both tumor promoting and suppressive activities, this study aims to further elucidate the EphA2 signaling mechanisms.

In the non-canonical signaling, high matrix rigidity promotes phosphorylation of EphA2 on S897, which leads LYN kinase binding and activation. Subsequently, LYN promotes TWIST1 localization to the nucleus and ultimately drives EMT. In contrast, in the EphA2 canonical signaling, ephrinA1-Fc ligand has been found to decrease S897 phosphorylation and LYN activation induced by ECM rigidity. This EphA2 canonical signaling thus blocks the stiffness-induced pathways. Therefore, this project hypothesizes that EphA2 can be one of the key mediators in the crosstalks between biochemical and mechanical cues in the tumor environment. EPHA2 possesses both tumor promoting and suppressive activities and plays a potential key mediator in the crosstalks between biochemical and mechanical cues during cancer development. At high ECM rigidity, EPHA2 non-canonical signaling axis can lead to EMT and breast cancer invasion and metastasis, while the binding of EphrinA1 ligand can block stiffness-induced EMT and invasion.

Defining the organ-specific roles of autophagy in breast cancer metastasis

Doglioni G., Encarnación Rosado J., Dufresne S., and Towers C.
Salk Institute for Biological Studies, La Jolla, CA 92037

Metastasis to distant organs is the main cause of death in breast cancer patients. And yet it is a highly inefficient process - many cancer cells invade and circulate, but few successfully colonize new sites. Organotropism, the preference for specific metastatic sites, is shaped by permissive niches influenced by the primary tumor. During dissemination, cancer cells adapt to the changing environments, but once at a distant organ, they require specific traits to grow, suggesting that the microenvironment imposes distinct dependencies. Uncovering these dependencies could reveal therapeutic opportunities to target metastasis in an organ-specific manner. I hypothesize that organotropic traits can be identified by profiling the intracellular and surface proteins of metastases across different organs. By modulating these proteins, we can determine which are essential for organ-specific metastatic growth. Autophagy regulates up to 70% of proteins within a cell, therefore I tested whether impairing autophagy could affect metastasis formation. Interestingly, my preliminary data show that metastases to certain organs, such as the liver, present with decreased autophagy activity and give rise to autophagy-independent metastasis, while lung metastasis growth is autophagy-dependent. However, how the secondary organ microenvironments shape specific dependencies for autophagy is unknown. By leveraging organ-specific genetic screenings with multi-omics analysis, I therefore aim to identify organotropic dependencies of breast cancer metastases that can be exploited to uncover new targeted therapies for metastatic patients, which are, up to now, uncurable.

Harnessing the tumoricidal activity of microglia in glioblastoma

Anais Elewaut, Aaron Liu, Matthew Miller, Lizmarie Garcia, and Susan Kaech
Salk Institute for Biological Studies

Glioblastoma (GBM) is one of the most aggressive primary brain tumors, with a median survival of just 12–15 months. Despite decades of research, current treatment options remain largely unchanged, underscoring the urgent need for novel therapeutic strategies. While immunotherapies have revolutionized treatment for various cancers, GBM remains largely resistant. This failure highlights a major gap in our understanding of which immune responses and cell types are capable of mediating effective anti-tumor activity in GBM. Our research aims to harness tumor-associated macrophages (TAMs), specifically microglia, the brain's resident macrophages, as a therapeutic axis in GBM. Contrary to the prevailing focus on their immunosuppressive roles, recent findings, including our own, reveal that microglia can exert protective, anti-tumor functions. We have shown that IFN-_–licensed microglia adopt an AXL/MER-driven phagocytic program critical for the efficacy of anti–CTLA-4 therapy in a mesenchymal GBM model. Remarkably, microglia were capable of killing GBM cells independently of T cell cytotoxicity, making microglia prime candidates for overcoming immune evasion in tumors with low T cell infiltration. To translate these findings, we will first develop a spatial, transcriptional, and temporal atlas of AXL^{+}/MHC II^{+} microglia using syngeneic mouse models and human GBM samples. By applying an innovative proximity labeling approach, we will identify key cellular interaction partners within these "phagocytic neighborhoods." This will allow us to map protective or tumoricidal microglia based on their direct cellular interactions *in situ* and identify ligand-receptor networks that regulate these niches. Second, we will define the molecular pathways that activate and sustain microglial phagocytosis and investigate how GBM cells suppress these functions. Together, these objectives aim to elucidate, potentiate, and harness TAMs, turning GBM from an immunologically 'cold' tumor into one susceptible to coordinated T cell and microglia attack

Engineering of ROR1- or ROR2-expressing T cells enhances proliferation, migration, and invasion to improve CAR-T anti-tumor efficacy

Daniel J. Elson PhD, Aaron M. Benedek, George F. Widhopf II PhD, and Thomas J. Kipps MD PhD
Department of Medicine, Center for Novel Therapeutics, Moores Cancer Center, University of California, San Diego, La Jolla, CA, USA

Chimeric antigen receptor T cell (CAR-T) efficacy in solid and hematologic malignancies is limited by suboptimal T cell engraftment, trafficking, persistence, and tumor microenvironment (TME)-mediated suppression. We hypothesized that co-opting Wnt5a–ROR1/2 signaling in T cells could enhance multiple hallmarks of CAR-T function, including proliferation, homing, invasion, and oxidative stress resistance. Primary human T cells from healthy donors, which express endogenous Wnt5a, were transduced with lentiviral vectors encoding ROR1 or ROR2 (or GFP-luciferase control) and analyzed for signaling, transcriptomic changes, and functional attributes *in vitro* and *in vivo*.

Virtual Inference of Protein-activity by Enriched Regulon analysis (VIPER) of bulk RNA-seq demonstrated that ROR1- or ROR2-expressing T cells, compared to GFP-expressing T cells, exhibited highly significant enrichment of ERK1/2 transcriptional effectors ELK1 and MYC, and increased target-gene expression for NRF2 and NF-κB, consistent with enhanced NRF2 and NF-κB activity. Gene set enrichment analysis revealed up_regulation of pathways associated with proliferation and growth, including E2F target gene signatures, G2/M checkpoint transition, and mTORC1 signaling. Immunoblotting confirmed increased phosphorylation of NF-κB p65 and ERK1/2, indicating that ROR1 or ROR2 expression in normal T cells activates downstream signaling similar to that observed in cancer cells.

Functionally, normal T cells and CAR_T cells expressing ROR1 or ROR2 showed 3–4_fold increased expansion following activation in IL-7/IL-15, enrichment of a T stem cell memory (Tscm) gene signature, and higher frequencies of $CCR7^{+}CD62L^{+}CD45RA^{+}$ and $CCR7^{+}CD62L^{+}CD45RA^{+}$ subsets. ROR1/2 expressing T cells displayed reduced baseline mitochondrial superoxide, consistent with enhanced NRF2_mediated oxidative stress tolerance. Moreover, ROR1_ or ROR2_ modified T cells demonstrated increased CXCL11_directed migration and Matrigel invasion, both abrogated by ROR1_specific (zilovertamab) or ROR2_specific (6E6_116) monoclonal antibodies, indicating pathway dependence. Collectively, these findings show that enforced ROR1 or ROR2 expression in T cells engages canonical Wnt5a–ROR1/2 signaling to enhance proliferation, Tscm_like features, chemokine_directed migration, invasion, and oxidative stress resilience, providing a framework for engineering next_generation CAR_T cells with improved fitness and tumor_infiltrating capacity in Wnt5a_rich TMEs.

Investigation into the role of polyamines and polyamine-binding proteins in cancer metabolism

Emily Fennell[1], Qijia Wei[2], Mack Reynolds[1], Appaso Jadhav[2], William Brothers[3], Gene Yeo[3], Christopher Parker[2], and Christian Metallo[1]
[1]Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA
[2]Department of Chemistry, Scripps Research Institute, La Jolla, CA
[3]Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA

Since their discovery, polyamines (putrescine, spermidine, and spermine) have been described in a variety of cellular functions, including DNA binding and protection, transcription, protein synthesis, glycolysis, and autophagy. Polyamines are central to numerous metabolic pathways and their rate-limiting synthetic enzyme (ornithine decarboxylase 1; ODC1) is overexpressed in some cancers. In 1992, ODC1 was identified as a bona fide c-MYC target gene, further implicating the role of polyamines in cancer cell growth. Difluoromethylornithine (DFMO), an inhibitor of ODC1, has shown some efficacy in clinical trials, further validating polyamine metabolism as a potential anticancer target. However, further research is essential to identify the impacts of polyamine depletion on metabolic function, as well as additional metabolic vulnerabilities that arise following ODC1 inhibition. In this project, we utilize a chemical proteomics-based approach to identify key polyamine-protein interactions using polyamine-based functionalized probes. Stable isotope tracing will also be employed in triple-negative breast cancer cells using 13C6-glucose and 13C5-glutamine to demonstrate alterations in metabolic flux in central carbon, amino acid, and lipid metabolism following ODC1 inhibition or polyamine supplementation. This dual approach will enable the identification of polyamine-regulated metabolic pathways and novel metabolic vulnerabilities for their exploitation as anticancer targets.

Predicted transcriptional activation domains are widespread in fusion oncoproteins

Eduardo Flores[1], Richard Kriwacki[2], and Lucia Strader[1]
[1]Department of Plant Biology, Salk Institute, La Jolla, CA, USA
[2]Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, TN, USA

Fusion oncoproteins (FOs) are created by chromosomal translocations and often act as key drivers of cancer. One subset produces abnormal transcription factors, typically formed by the fusion of an intrinsically disordered region (IDR) from one parent protein to a folded DNA- or chromatin-binding domain. Many IDRs, sequences that lack a well-defined tertiary structure, contain transcriptional activation domains responsible for recruiting transcription factor machinery. Aberrant fusion oncoproteins can improperly recruit transcriptional and chromatin-remodeling coactivators, leading to abnormal gene expression. Here, we take 3,174 curated fusion oncoprotein sequences from sources at St. Jude Children's Research Hospital and from The Cancer Genome Atlas, and use our transcriptional activation domain activity neural network (TADA) to identify disordered regions predicted to function as transcriptional activation domains. We identified predicted activation domains within disordered regions in more than 3,000 FOs. About 1,500 of these FOs are associated with B-cell lymphoblastic leukemia, breast invasive carcinoma, or lung squamous cell carcinoma, while others are linked to additional cancer types. Clinically, more than 2,000 FOs with predicted activation domains have been observed in multiple patients. These results suggest that transcriptional activation domains are widespread and clinically relevant features of fusion oncoproteins. Future work will validate these predicted activation domains experimentally and determine how their sequence features modulate activity.

Tumor necrosis factor blockade uncouples lung hyperplasia from cancer progression in aging mice

Kefei Gao[1,2], Himani Sharma[3], Isaac Bell[3], Farrah Kheradmand[4], and Silke Paust[1,2,3]

[1]Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA 92037

[2]The Skaggs Graduate Program, The Scripps Research Institute, La Jolla, CA 92037

[3]The Jackson Laboratory for Genomic Medicine, Farmington, CT 06032

[4]Baylor College of Medicine, Houston, TX 77030

Solid tumors can evolve for years to decades in patients, yet many mouse models rely on juvenile mice and compress tumorigenesis into weeks, limiting therapeutic separation of early epithelial lesions from malignant progression. In an airway epithelium-specific, genetically engineered model of adenosquamous lung cancer that progresses from hyperplasia to tumors and metastasis on age-appropriate timelines, we uncouple hyperplasia from cancer by blocking tumor necrosis factor (TNF). Loss of mature NK cells accelerates tumor onset and incidence; paradoxically, depleting NK cells in this setting suppresses tumors without eliminating hyperplasia, implicating dysregulated immature NK cells as drivers. TNF-blockade during progression prevents tumor, but not hyperplasia development and normalizes NK maturation. Our findings support TNF-blockade as a stage-specific strategy to intercept lung cancer progression.

ULK1/2 inhibitors that degrade ATG13 effectively target KRAS-mutant cancers

Patrick M. Hagan[1], Huiyu Ren[1], Sonja N. Brun[3], Fabiana I. A. Layng[1], Dhanya R. Panickar[2], Lester J. Lambert[1], Maria Celeridad[1], Ranajit Das[1], Li Ling[1], Yijuan Zhang[1], Allison S. Limpert[1], Douglas J. Sheffler[2], Cosimo Commisso[1], Reuben J. Shaw[3], and Nicholas D. P. Cosford[1]

[1]Cancer Metabolism and Microenvironment Program, Center for Therapeutics Discovery, NCI Designated Cancer Center, Sanford Burnham Prebys Medical Discovery Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037

[2]Cancer Genome and Epigenomics Program, Center for Therapeutics Discovery, NCI Designated Cancer Center, Sanford Burnham Prebys Medical Discovery Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037

[3]Department of Molecular and Cell Biology, Salk Institute for Biological Studies, San Diego, La Jolla, CA 92037

KRAS mutations drive tumorigenesis in multiple cancer types, including lung and pancreatic cancer. Autophagy is a cell survival pathway that supports tumor growth under metabolic stress and has been proposed to be a potential therapeutic avenue specifically in KRAS mutant cancers. The Unc-51-like ATG-activating kinases 1 and 2 (ULK) initiate the earliest regulated steps of autophagy and are the only protein kinases in the canonical autophagy pathway, thus making them attractive therapeutic targets for KRAS mutant tumors. We show here that genetic depletion of ULK1 or ATG13, core components of the ULK1 complex, in KRAS mutant lung and pancreatic cancer cell lines results in growth inhibition. Previously, we developed small molecule ULK1 inhibitors that not only inhibit ULK kinase activity but also induced the degradation of other core members of the ULK complex including ATG101 and ATG13. Therefore, we developed a high-throughput screening (HTS) assay in which ATG13 was HiBiT-tagged in KRAS mutant lung cancer cells to evaluate ULK inhibitors for ATG13 degradation. Using this approach, we discovered a lead ULK inhibitor, SBP-1750, that potently inhibited ULK activity, promoted robust ATG13 degradation, impaired ATG, and induced KRAS mutant cancer cell death. Studies in a KRAS-mutant orthotopic syngeneic pancreatic cancer model show that oral treatment with SBP-1750 significantly reduced tumor growth. Pharmacokinetic analysis of SBP-1750 indicates favorable drug exposure and pharmacodynamic analysis confirms ATG13 degradation *in vivo*, mirroring *in vitro* results. Finally, immunohistochemical staining of orthotopic pancreatic tumors reveals a significant increase in $CD4^+$ and $CD8^+$ T cell infiltration upon treatment, suggesting that SBP-1750 enhances anti-tumor immunity. These findings support further development of SBP-1750 as a novel ATG-targeting cancer therapy.

Catestatin peptide mediated rewiring of oncogenic pathways to suppress melanoma and overcome drug resistance

Satadeepa Kal[1,3*], Suborno Jati[2], Kechun Tang[1], Nicholas J.G. Webster[3,4], Angelo Corti[5], and Sushil K. Mahata[3,4*]
[1]Veterans Medical Research Foundation, San Diego, CA, USA
[2]Department of Neurosciences, University of California, San Diego, CA, USA
[3]Department of Medicine, University of California, San Diego, CA, USA
[4]VA San Diego Healthcare System, San Diego, CA, USA
[5]IRCCS San Raffaele Scientific Institute, San Raffaele Vita-Salute University, Milan, Italy

Melanoma remains one of the most aggressive and therapy-resistant cancers, emphasizing the need for innovative therapeutic strategies. While small-molecule and immune checkpoint inhibitors are prevalent, they do not work for everyone, necessitating new interventions to curb the disease. In this context, we report a peptide-based approach as a potential therapeutic, describing, for the first time, the involvement of the Catestatin (CST) peptide in carcinogenesis, with melanoma identified as an unexplored and therapeutically relevant context. The expression and role of CST, a Chromogranin A (CgA)-derived peptide with immunomodulatory and reparative properties in skin injury, led us to examine its connection to melanoma. Analysis of human melanoma tissues revealed decreased CST expression with advancing disease stage, suggesting a potential tumor-suppressive function. Restoration of CST in patient-derived melanoma cells and established melanoma cell lines induced apoptosis and suppressed proliferation and migratory capacity, sparing the normal skin fibroblasts, thus indicating tumor-selective activity. *In vivo*, CST administration significantly reduced tumor growth and tumor weight in the B16F10 melanoma mouse model. A deeper analysis of mechanistic insights reveals altered transcriptomic profiles in CST-treated melanoma cells and tumors. Downregulation of pathways involved in hypoxia signaling, extracellular matrix remodeling, epithelial-to-mesenchymal transition (EMT), and stress-adaptive responses, key drivers of melanoma invasion and progression, is observed. Consistent with these findings, CST suppressed several mediators of tumor progression. CST also suppressed oncogenic progression in Vemurafenib-resistant A375 cells, accompanied by the downregulation of multiple resistance-associated genes. While these findings establish Catestatin as a novel regulator of melanoma growth and therapeutic resistance, further mechanistic details and its therapeutic potential are being investigated.

Disrupting complex sphingolipid metabolism enhances EGFR inhibition in cancer

Samuel Kerk[1], Casie Kubota[1,2], Tiona Truong[1], Chris Murray[1], Karl Wessendorf-Rodriguez[1], Mo Ruchhoeft[1], Dannielle Engle[2], Reuben Shaw[1], and Christian Metallo[1]
[1]Molecular and Cellular Biology Laboratory
[2]Regulatory Biology, Laboratory Salk Institute for Biological Studies, La Jolla, CA

The role of the epidermal growth factor receptor (EGFR) is well-defined in cancer biology and EGFR inhibitors (EGFRi) have been deployed against numerous tumor types for decades. However, resistance is common. Thus far, mechanistic studies have largely focused on changes to the receptor itself or the activation of compensatory signaling pathways in the cancer cell. Less is known about how the underlying lipid metabolism of a cancer cell influences EGFR signaling and response to EGFRi, despite the receptor residing in and interacting with the lipid bilayer. Here, we demonstrate how a critical node in complex sphingolipid metabolism can be leveraged for improved EGFRi in cancer.

Complex sphingolipids are a major lipid class in the cell and consist of numerous subspecies such as gangliosides, globosides, and sphingomyelin. Synthesis of many of these molecules begins with the addition of a hexose sugar headgroup to a ceramide backbone by the enzyme UDP-glucose ceramide glucosyltransferase (UGCG). In other contexts, UGCG has been shown to influence membrane protein activity.

As a proof of concept, we broadly disrupted complex sphingolipid metabolism using the UGCG inhibitor eliglustat. Interestingly, colon, lung, and pancreas cancer cell lines cultured in eliglustat were more sensitive to the EGFRi gefitinib. Next, we treated *ex vivo* organotypic slices from transgenic murine lung and pancreas tumor models with eliglustat and surprisingly discovered increased pEGFRY1068, which was abrogated by combination therapy with gefitinib. Additionally, dual tracing of glucose and serine into complex sphingolipids and subsequent mass spectrometry analyses revealed decreased flux through UGCG into downstream complex sphingolipids. In particular, the abundance of the ganglioside GM3 decreased. This is significant given previous work demonstrating GM3 negatively regulates EGFR signaling. Therefore, we propose a mechanism by which loss of certain complex sphingolipids paradoxically increases EGFR signaling, in effect priming cancer cells for combination therapy with EGFRi. More fundamentally, these findings illustrate targeting cell metabolism is an effective avenue for improving current cancer therapies.

Targeting trans-Golgi network acidification as a therapeutic vulnerability in pancreatic cancer

Elham Khosrowabadi[1], Charles Fisher[2], Koen Galenkamp[1], Cheska Marie Galapate[1], Shea Grenier Davis[1], Susanne Heynen-Genel[2], and Cosimo Commisso[1]
[1]NCI-Designated Cancer Center, Sanford Burnham Prebys Medical Discovery Institute, 10901 N. Torrey Pines Road, La Jolla, CA 92037, USA
[2]The Conrad Prebys Center for Chemical Genomics (CPCCG), Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA

Pancreatic ductal adenocarcinoma (PDAC) is projected to become the second leading cause of cancer-related deaths in the United States by 2030 and has a 5-year survival rate of approximately 13%, underscoring the urgent need for new therapeutic strategies. PDAC undergoes profound metabolic reprogramming that supports survival in nutrient- and oxygen-poor microenvironments. Preferential reliance on glycolysis leads to excessive proton (H^+) production, which disrupts cytosolic pH homeostasis and compromises cell viability. We identify an organelle-based buffering strategy in PDAC whereby excess cytosolic H^+ is buffered within intracellular compartments, with the trans-Golgi network (TGN) functioning as a critical proton "sink." This homeostatic mechanism is largely absent in normal pancreatic epithelial cells, revealing a cancer-selective vulnerability.

Mechanistically, the TGN-localized Na^+/H^+ antiporter NHE7 is required to maintain cytosolic pH in PDAC cells. Genetic or pharmacologic inhibition of NHE7 induces cytosolic acidification and selectively compromises PDAC cell viability while sparing normal cells. These effects are recapitulated in mouse models of PDAC. Analysis of patient datasets shows that TGN-localized ion transporters are frequently overexpressed in PDAC, and their elevated expression correlates with worse overall survival, supporting the clinical relevance of Golgi pH regulation in tumor biology.

To therapeutically exploit this metabolic dependency, we developed a TGN-targeted pH biosensor compatible with high-throughput screening of small-molecule libraries to identify selective inhibitors of Golgi acidification. This platform, coupled with secondary and mechanistic validation assays, nominates Golgi-selective compounds that disrupt organellar pH homeostasis and induce cancer-selective cell death. Together, these findings establish Golgi acidification as a targetable metabolic vulnerability in PDAC and provide a scalable strategy to discover first-in-class organelle-selective therapeutics.

Malic enzyme inhibition as a strategy to exploit metabolic reprogramming in cancer: A structure-based approach

Ben Krinkel[*1], Yuliana Yosaatmadja[1], Mark D. Slayton[2], Jin Heon Jeon[2], Jack Copping[1], Jack Flanagan[1], Sofia D. Merajver[1], Christopher Squire1, and Kerry Loomes[1]
[1]School of Biological Science, University of Auckland, New Zealand, 1010
[2]Michigan Medicine, University of Michigan, Ann Arbor, MI, United States of America, 48109

Background: Cancer remains a global health challenge and urgently requires therapies that improve clinical outcomes and patient quality of life. Tumor cells commonly reprogram metabolism to sustain proliferation and survival, notably by increasing glutamine dependence and upregulating mitochondrial malic enzyme 2 (ME2), which produces pyruvate and NAD(P)H. ME2 is overexpressed in pancreatic, melanoma, and lung cancers, making it a compelling therapeutic target that remains underexplored.

Methods: Recombinant human ME1, ME2, and ME3 were expressed and kinetically characterized in the presence and absence of inhibitors. We solved a complete set of X-ray crystal structures for the three isoforms with a potent malic enzyme inhibitor bound, revealing ligand and metal coordination. These structures guided virtual screening of a curated library of up to 14 million drug-like and fragment molecules to identify novel malic enzyme inhibitors. Biochemical hits were advanced to cellular assays to evaluate efficacy; lead compounds NPD-389 and FLA were tested for antiproliferative activity in melanoma and triple-negative breast cancer cell lines.

Results: Kinetic analyses show distinct activity and inhibition patterns across ME isoforms. X-ray crystallography visualized the potent, isoform-nonselective inhibitor NPD-389 bound in a metal-coordinating mode and captured an unexpected malic enzyme conformation. Structure-based virtual screening against this conformation identified multiple novel scaffolds with biochemical inhibitory activity. One scaffold, FLA, exhibited a unique selectivity profile and was found bound to ME2 in a previously cryptic pocket that rearranges upon ligand engagement and sits adjacent to the NAD+ cofactor and malate-binding site. In a panel of melanoma and triple- negative breast cancer cell lines, NPD-389 and FLA reduced proliferation in most models, supporting their potential as chemical leads for further development..

Conclusions: These results establish mitochondrial malic enzymes, particularly ME2/ME3, as tractable targets for cancer therapy. Structural elucidation of NPD-389's metal-binding mode and of FLA's cryptic/allosteric binding pocket provides a robust foundation for rational optimization of potency and isoform selectivity. Selective ME inhibitors—either isoform-specific or mitochondrially targeted—have potential as adjuncts to checkpoint inhibitors or chemotherapy, enabling more effective, metabolism-directed cancer treatment.

Evaluating the efficacy and mechanism of action of seriniquinone against pediatric neuroblastoma cell lines

Erin Yamaoka, Disha Surana, Sriprada Kattamuri, Tushara Kumar, Carla Sampaio, William Fenical PhD, Peter E. Zage M.D. PhD
Department of Pediatrics, University of California San Diego, La Jolla, CA
Department of Oceanography and Pharmaceutical Science, Center for Marine Biotechnology and Biomedicine, Scripps Institute of Oceanography, University of California, San Diego, La Jolla, CA

Background: Children diagnosed with neuroblastoma (NB) face poor prognosis despite aggressive multimodal therapies, highlighting the urgent need for effective and targeted treatments. Natural products derived from microbial sources like marine actinomycetes are promising novel anticancer agents. Seriniquinone, a quinone-based compound originally isolated from a Serinicoccus species, has demonstrated potent anticancer activity across a variety of tumor types. It exerts its effect by targeting dermcidin, a peptide associated with cancer cell survival and proliferation, inducing autophagocytosis followed by apoptosis. Given its unique mechanism of action and prior efficacy, we hypothesize that seriniquinone may represent a novel and selective therapeutic option for pediatric neuroblastoma. Methods: Techniques used included cell culture and maintenance, quantification via cell counting, CCK8 viability assays, and Western Blot analysis. Synthetic seriniquinone was tested by preparing 96-well plates and 10 cm plates with different neuroblastoma cell lines, including MYCN-amplified and non-amplified subtypes. Cells were treated with a control and various increasing concentrations of seriniquinone to perform CCK-8 cell viability assays. Western Blots analysis of whole cell lysates is used to evaluate protein expression changes and seriniquinone's effect on GADPH and PARP, and to detect apoptotic markers such as Caspase-3. Results: Treatment of various MYNC-amplified and MYCN non-amplified NB cell lines shows that Seriniquinone inhibits cell growth, found with CCK-8 viability assays. Microscopy images of NGP-14 and TR14 cell lines show cell rounding, detachment and death compared to the healthy monolayers in the DMSO treated controls. Existing preliminary data from the Fenical lab regarding the compound's effect on melanoma revealed downregulation of dermcidin and N-Myc in treated cells, from Western Blot analysis. Control conditions showed no such changes. Conclusions: Our results support the efficacy of seriniquinone as a potent therapeutic agent for pediatric NB. We plan to further analyze the mechanism of action of seriniquinone on pediatric NB cells through additional Western Blot analyses for mechanism of action. Ultimately, we hope these findings lay the groundwork for further preclinical validation and support future clinical investigation, including the potential for an IND application.

Examining mechanism of resistance to RMC-6236 through the S1P receptor pathway in pancreatic ductal adenocarcinoma

Elysia Kuo[1], Casie S. Kubota[1,2], Vasiliki Pantazopoulou[1], Kassidy Curtis[1], Isabel Vasquez[2], Christian M. Metallo[2], and Dannielle D. Engle[1]

[1]Regulatory Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA

[2]Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA

Pancreatic ductal adenocarcinoma (PDAC) is projected to be the second leading cause of cancer-related deaths in the U.S. by 2040 with a 5-year survival rate only about 13%. Despite advances in the field, treatment options remain limited with surgery and chemotherapy being the primary options for care. Recently, the successful development of RAS inhibitors, Sotorasib and Adagrasib, against the KRASG12C mutation has opened the line of development for more RAS-targeted therapies. RMC-6236 is a small molecule pan-RAS inhibitor currently in clinical trials. Previous research has shown in patients treated with Sotorasib and Adagrasib that while initially effective, resistance to RAS inhibition is eventually acquired in most patients. However, the mechanism of resistance to RMC-6238 remains to be elucidated. To examine this, we have generated and characterized two RMC-6236 resistant mouse PDAC 2D cell lines. Through qPCR analysis, we have found that there is an increase in gene expression of the sphingosine-1-phosphate receptor (S1PR) family, in particular S1PR-5, in the RMC-6236 resistant cells. We hypothesize that signaling through S1PR-5 could be a mechanism of resistance, as the S1PR family are also capable in activating proliferative pathways common in cancer such as AKT, PI3K, and ERK signaling pathways. This work will help in understanding the role of S1PR-5 in acquiring resistance to RMC-6236 and potentially identify a target to combat the development of RMC-6236 resistance.

MICAL2 promotes metabolic reprogramming in pancreatic ductal adenocarcinoma

Mia MacDonald, MD[1], Guillem Lambies-Barjau, PhD[2], Ponmathi Paneerpandian, PhD[1], Herve Tiriac, PhD[1], Cosimo Commisso, PhD[2], and Andrew Lowy, MD[1]
[1]University of California San Diego, Moores Cancer Center, Department of Surgery, La Jolla, CA
[2]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA

MICAL2 is a super-enhancer–associated gene that amplifies oncogenic KRAS signaling to promote pancreatic ductal adenocarcinoma (PDAC) growth and metabolic adaptation. Our prior work demonstrated that loss of MICAL2 impairs macropinocytosis and upregulates gene ontology pathways involved in lipid and cholesterol biosynthesis. However, whether MICAL2 directly regulates other metabolic programs supporting nutrient acquisition and lipid metabolism remains unclear. MICAL2 was silenced by siRNA in ASPC1 human PDAC cells. Polar metabolomics quantified changes in extracellular metabolite abundance 24–72 hours after MICAL2 knockdown (M2KD). Stable isotope tracing with 13C-glucose and 13C-glutamine was performed to assess carbon incorporation into amino acids and TCA cycle intermediates. Cholesterol and lipid metabolism genes altered more than twofold on RNA-seq following M2KD were validated by qPCR in ASPC1 and KPC46 cells. Autophagic flux was evaluated using LC3B turnover assays. MICAL2 knockdown increased glutamate and lactate secretion 48-72 hours after silencing and enhanced glutamine uptake at 72 hours. Intracellular pools of both essential and non-essential amino acids were broadly reduced in M2KD cells. Total citrate abundance was significantly decreased despite preserved downstream TCA intermediates. Abrogation of MICAL2 also downregulated genes involved in cholesterol homeostasis and lipid trafficking (LIPA, NCEH1, PCSK9, CAV1, ABCG1) and reduced LC3B-II turnover, consistent with decreased autophagic flux. Loss of MICAL2 is associated with reduced intracellular pools of essential and non-essential amino acids and decreased autophagic flux, consistent with impaired nutrient acquisition. Reduced citrate abundance in M2KD cells, with preservation of downstream TCA intermediates, suggests maintained TCA cycle activity with potential shunting of citrate toward biosynthetic pathways such as lipid synthesis. Consistent with this possibility, we observed downregulation of genes involved in cholesterol uptake, catabolism, and trafficking (LIPA, NCEH1, PCSK9, CAV1, and ABCG1), suggesting reduced reliance on extracellular lipid acquisition. Together, impaired autophagy and upregulated *de novo* fatty acid synthesis in M2KD cells may create metabolic vulnerabilities that could be therapeutically exploited. Future non-polar metabolite tracing studies will be required to assess how MICAL2 impacts lipid synthesis and utilization.

Macrophage metabolism supports immunomodulatory ECM that drives tumor progression

Cray Minor[1], Kevin Tharp[1], Allen Lee[1], Isabel Sakowicz[1], Danielle Grotjahn[2], Luke Wiseman[2], Samantha Oviedo[2], Colin Anderson[3], Angelo D'Alessandro[3], and Julie Hanes[3]
[1]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037
[2]The Scripps Research Institute, La Jolla, CA 92037
[3]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado, Aurora, CO 80045

Breast tumors are characterized by low T-cell infiltration, which underpins their poor responsiveness to immune checkpoint blockade (ICB). The Luminal A subtype exhibits the lowest immune cell infiltration, and its progression is accompanied by progressive fibrosis and extensive macrophage accumulation. Tumor-associated macrophages (TAMs) play heterogeneous roles in the tumor microenvironment (TME), including direct contributions to impaired ICB responses; however, the mechanisms by which they do so remain unclear. We identified a subset of human TAMs exhibiting an IL17F-driven Activating Transcription Factor 4 (ATF4) stress response that promotes metabolic rewiring to support a collagen-synthetic phenotype, to suppress T Cell infiltration. We hypothesize that targeting this immunosuppressive macrophage axis could restore ICB sensitivity in breast cancer. In vitro, through ATF4, IL17F induces a 2.5-fold increase in P5CS and a >10-fold elevation in 1-carbon–derived serine-to-glycine intermediates, thereby licensing a macrophage collagen-synthetic state. Pharmacologic inhibition of ATF4 by ISRIB ameliorates these phenotypes. In an orthotopic PyMT-Ova murine breast cancer model, macrophage-specific genetic deletion of ATF4 reduces Collagen VI deposition by ~40% and increases T-cell infiltration within the TME compared to wildtype controls. Collagen-synthetic macrophages also downregulate costimulatory molecules, limiting immune activation, in addition to physically restricting adaptive immune cells by stromal fibrosis accumulation. Together, our findings reveal a macrophage trajectory that is both pro-fibrotic and anti-immunogenic, delineating a therapeutically actionable axis by which targeting macrophage ATF4-dependent metabolic adaptation may restore immune accessibility and improve ICB outcomes in breast cancer.

A lipid metabolic checkpoint in the progression to lung adenocarcinoma

Christopher W. Murray[1], Hector M. Galvez[1], Ruoxi Wang[1], Karl A. Wessendorf-Rodriguez[1,2], Sandy Lee[3,4], Fernando Lopes[1], Aubrey N. Michi[1], Jose A. Sandoval[1], Antonio F.M. Pinto[5], Maureen L. Ruchhoeft[1], Yuning J. Tang[6], Nianfei Xian[1], Monte M. Winslow[6,7], Michael La Frano[2], David Shackelford[3,4], Christian M. Metallo[1,2], Alan Saghatelian[8], and Reuben J. Shaw[1]
[1]Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA
[2]Mass Spectrometry Core, Salk Institute for Biological Studies, La Jolla, CA 92037
[3]Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093
[4]Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305
[5]Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305
[6]Division of Pulmonary and Critical Care Medicine, Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA 90095
[7]Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles, CA 90095
[8]Clayton Foundation Laboratories for Peptide Biology, Salk Institute for Biological Studies, La Jolla, CA 92037

Lung cancer is the leading cause of cancer-related deaths worldwide. Lipid metabolism plays a key role in tumorigenesis, fueling membrane biogenesis and shaping membrane dynamics, supporting energy storage, and generating signaling molecules. We and others have previously demonstrated a critical supportive role for *de novo* fatty acid synthesis in lung cancer. However, many lipid metabolic processes remain to be functionally defined in this disease context. To address this significant gap, we conducted a lipid metabolism-targeted CRISPR/Cas9 screen in a genetically engineered mouse model of oncogenic KRAS-driven lung adenocarcinoma. Surprisingly, among the most potent tumor suppressor genes, we noted Cpt2 (carnitine palmitoyltransferase 2), which operates as part of the carnitine shuttle to mediate the import of long-chain fatty acids into the mitochondrial matrix for β-oxidation. Consistent with its canonical function, inactivation of Cpt2 in lung tumors impaired the oxidation of palmitate and resulted in the accumulation of long-chain acylcarnitines and fatty acids. We also noted a global accumulation of glycerolipids, accompanied by significant decreases in lysophospholipid and phospholipid abundance, suggesting a shift away from membrane remodeling and toward lipid storage. Notably, this was accompanied by reductive shifts in glutathione and ascorbate pools, suggesting enhanced capacity to buffer oxidative stress. At the transcriptional level, Cpt2 deficiency resulted in elevated expression of a series of targets of the central orchestrator of lipid catabolism, PPARα. Strikingly, we observed an enrichment of tumors in the Cpt2¬deficient setting that express multiple transcription factors involved in lipid homeostasis in hepatocytes, including HNF4α, HNF1α, and CREB-H, in addition to markers of gastrointestinal identity. These transcriptional changes mirror features of transition states in the progression towards lung adenocarcinoma, suggesting that lipid metabolic alterations may underlie disease progression. Finally, we determined that there exist multiple proximal genes in the long-chain fatty acid oxidation pathway that exert comparable tumor-suppressive activity and, upon inactivation, result in transcriptional and metabolic features that phenocopy Cpt2 deficiency.

Peptidoglycan remodeling in *Enterococcus faecium* enhances cancer immunotherapy via NOD2 activation

Anh PN. Nguyen[1], Pavan K. Chodisetti[1], Kyong T. Fam[1], Adrianna Turner[1], and Howard C. Hang[1,2]

[1]1Department of Immunology and Microbiology, Scripps Research, La Jolla, CA, 92037

[2]Department of Chemistry, Scripps Research, La Jolla, CA, 92037

The gut microbiota is increasingly recognized as a key regulator of host physiology and therapeutic outcomes, including cancer immunotherapy. Notably, enrichment of specific *Enterococcus* species has been observed in immune checkpoint inhibitor (ICI)-responsive patients, yet the molecular mechanisms by which individual microbes modulate ICI efficacy remain poorly defined. Here, we investigated whether cell wall remodeling in vancomycin-resistant *Enterococcus faecium* (VREfm) influences ICI outcomes. Through bioinformatic analysis of publicly available clinical datasets comprising over 300 patients with multiple cancer types receiving ICI therapy, we identified secreted antigen A (*sagA*), encoding an NlpC/p60 peptidoglycan hydrolase, as a conserved core gene across all E. faecium strains and significantly associated with treatment response. In a murine B16F10 melanoma model, oral administration of VREfm in combination with ICI reduced tumor volume by ~2-fold and extended median survival by 10 days compared to ICI alone. Importantly, *sagA* was both necessary and sufficient to enhance ICI-mediated anti-tumor activity, supporting microbial enzymatic pathways as potential therapeutic adjuvants. VREfm supplementation also increased intratumoral infiltration of total and cytotoxic (Granzyme B^+) $CD8^+$ T cells, indicating enhanced anti-tumor immunity. Mechanistically, VREfm activated nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a pattern recognition receptor expressed in epithelial and myeloid cells. Markerless deletion of *sagA* abolished NOD2 activation *in vitro*, while chromosomal complementation at a neutral locus restored this activity. Consistently, the ICI-potentiating effect of VREfm was lost in Nod2-deficient mice, demonstrating a NOD2-dependent mechanism *in vivo*. Together, these findings identify bacterial peptidoglycan remodeling as a critical determinant of microbiota-mediated enhancement of cancer immunotherapy and highlight a targetable microbial pathway for rational design of microbiota-based cancer therapies.

Targeting MICAL2 sensitizes pancreatic cancer cells to gemcitabine and enhances therapeutic response

Ponmathi Panneerpandian, Kevin Gulay, Bharti Garg, Isabella Ng, Hervé Tiriac, and Andrew M. Lowy
Moores Cancer Center, Department of Surgery, University of California San Diego

Background: Pancreatic ductal adenocarcinoma (PDAC) remains a highly lethal malignancy, largely due to rapid development of resistance to standard chemotherapies such as Gemcitabine. Identifying molecular pathways that drive therapeutic resistance is essential to improve outcomes. Here, we investigate the role of MICAL2, a cytoskeletal regulator, in modulating drug sensitivity in PDAC.

Methods: Human and murine PDAC cell lines and patient-derived organoids were used to evaluate MICAL2 function in drug response. Inducible knockdown and overexpression systems were employed to manipulate MICAL2 levels. Drug sensitivity was assessed using real-time proliferation (CellCyte), cell viability, and colony formation assays following treatment. Cell cycle distribution was analyzed by flow cytometry. *In vivo* efficacy of MICAL2 targeting in combination with chemotherapy was evaluated using PDAC xenograft models.

Results: Inducible MICAL2 knockdown significantly increased sensitivity to gemcitabine across multiple models, including human and murine PDAC cell lines and patient-derived organoids. This was reflected by reduced proliferation (2.5-fold decrease, $p<0.001$), decreased viability (~50%, $p<0.005$), and reduced clonogenic survival (~65%, $p<0.0001$) compared to treatment alone. In contrast, MICAL2 overexpression conferred resistance, enhancing survival and colony formation under treatment. Mechanistically, combined MICAL2 knockdown and gemcitabine treatment induced marked cell cycle arrest, indicating reduced proliferative capacity. MICAL2 suppression also enhanced the efficacy of gemcitabine in combination with paclitaxel or the RAS(ON) inhibitor RMC-6236. *In vivo*, MICAL2 knockdown potentiated gemcitabine-mediated tumor growth inhibition.

Conclusions: MICAL2 is a key regulator of chemotherapy resistance in PDAC and a potential therapeutic target. Targeting the MICAL2 axis may enhance the efficacy of existing treatments and represents a promising strategy to overcome resistance and improve patient outcomes.

Peptide-delivered low dose IL-2 reprograms pancreatic cancer immunity to improve survival

In Hwan Park[1,3], Shawn Abeynaike[1,3], Ashley Martinez[1,3], Jonathan Cho[1,3], Charles Jurisaga[1,3], Daisuke Nishizaki[3], and Gregory Botta[1,2,3]
[1]Department of Medicine, Division of Hematology/Oncology, Cancer Biology and Signaling Program
[2]Department of Medicine, Division of Cellular & Regenerative Medicine
[3]Moores Cancer Center, University of California, San Diego, La Jolla

Metastatic pancreatic cancer (PC) has a 5-year survival of ~2% due to poor drug penetration, resistance to immunotherapy, and an immunosuppressive TIME characterized by dense desmoplasia, dysfunctional T cells, and enriched Tregs. Interleukin-2 (IL-2) can activate cytotoxic T cells, but high-dose (HD) IL-2 therapy causes severe systemic toxicities including CLS, hypotension, and renal failure. Strategies that enhance tumor-directed IL-2 activity while minimizing systemic toxicity are needed.

The tumor-penetrating CendR peptide iRGD binds αvβ3 integrins and induces neuropilin-1–dependent transcytosis of co-administered therapeutics. We hypothesized that iRGD could enhance intratumoral delivery of low-dose (LD) IL-2 to generate localized immune activation in pancreatic cancer. Orthotopic KPC pancreatic tumors were established in C57BL/6 mice. Tumor-bearing mice received intravenous iRGD (150 μg/mouse) with LD IL-2 (0.25 mg/kg) or HD IL-2 (25 mg/kg) three times weekly. Tumor growth, survival, immune activation markers, systemic cytokines, and lung histology were assessed. Patient-derived PDAC slice cultures were treated *ex vivo* to evaluate translational relevance.

iRGD + LD IL-2 significantly reduced tumor burden and improved survival compared with either agent alone (median survival 2.7 vs 0.7 months, $p<0.0001$). The combination enhanced CD8+ T-cell cytotoxic function without increasing CD8+ infiltration or proliferation. Mechanistically, tumors demonstrated increased phospho-STAT5 signaling, elevated Granzyme B expression, and increased cleaved Caspase-3, indicating enhanced T-cell–mediated tumor cell killing. CD8+ T-cell depletion abrogated the anti-tumor effect, confirming dependence on cytotoxic T cells. Importantly, iRGD + LD IL-2 did not induce systemic inflammation, cachexia, or CLS as measured by serum IL-6, TNF-α, and lung histology. Consistent increases in Granzyme B expression were observed in patient-derived PDAC slice cultures treated with iRGD + IL-2.

Tumor-targeted delivery of LD IL-2 using iRGD reprograms pancreatic cancer immunity by enhancing functional cytotoxic CD8+ T-cell activity while avoiding the systemic toxicities associated with HD IL-2. This strategy significantly suppresses tumor growth and prolongs survival in preclinical pancreatic cancer models. These findings support further clinical development of iRGD combined with low-dose IL-2 as a novel immunotherapeutic approach for pancreatic cancer.

Targeting myeloid metabolism to remodel the pancreatic tumor immune microenvironment

Austin D. Silva[1], Avary Baker[1], Mariam Mohagheghi[1], Ethan Smiggs[1], Dequina Nicholas[1], and Christopher J. Halbrook[1,2]
[1]Department of Molecular Biology and Biochemistry, University of California Irvine, Irvine CA 92697
[2]University of California Irvine Chao Family Comprehensive Cancer Center, Orange, CA 92686

Pancreatic ductal adenocarcinoma (PDA) is projected to become the 2nd most prevalent cancer-related death in the United States. The lethality of PDA is largely due to a lack of effective treatment options. A principal barrier in PDA treatment is the physiology of the tumors, characterized by a densely fibrotic stroma rich with immune cell infiltration. Most of these immune cells are immunosuppressive myeloid cells, including macrophages, polarized by environmental cues that dictate their function. This polarization has typically been thought of as pro-inflammatory or anti-inflammatory. In PDA, macrophages within the tumor, termed tumor-associated macrophages (TAMs), are strongly immunosuppressive, inhibiting both infiltration and activation of cytotoxic T cells. Further, TAMs have been shown to directly inhibit chemotherapy in PDA. Accordingly, targeting or reprogramming TAMs may sensitize pancreatic cancer to multiple treatment strategies.

We and others have shown that the polarization of macrophages to a pro- and anti-inflammatory state drives the adaptation of specific metabolic programming. Specifically, anti-inflammatory macrophages, including TAMs, preferentially use mitochondrial metabolism for bioenergetics. Accordingly, this provides a potential avenue to employ metabolic intervention to reprogram or eliminate pro-cancer TAM populations. To examine if targeting mitochondrial metabolism can impair the functional anti-inflammatory properties of macrophages, we have generated a model to genetically ablate a transcription factor required for ETC assembly in a myeloid-specific manner. Our preliminary data demonstrate that mice harboring defective oxidative metabolism in myeloid cells have an altered response to pancreatic inflammation. Moving forward with these models, we expect to use them to investigate the role of myeloid mitochondrial metabolism on pancreatic tumorigenesis, immune suppression in the pancreatic tumor microenvironment, and PDA response to therapy.

Fibulin-3 promotes tumor progression and microenvironment remodeling in CA19-9-expressing pancreatic ductal adenocarcinoma

Hyemin Song[1,2], Jasper Hsu[1], Satoshi Ogawa[1], Casie Kobuta[1], Jonathan Zhu[1], Xiaoxue Lin[1], Kristina L. Peck[1], Chelsea Bottomley[1], Kassidy Curtis[1], Elysia Kuo[1], Araceli Herrera Morales[1], McKenna Stamp[1], and Dannielle D. Engle[1]
[1]Salk Institute for Biological Studies, La Jolla, CA, USA
[2]Department of Biomedical Sciences, UC San Diego, La Jolla, CA, USA

Pancreatic ductal adenocarcinoma (PDA) is one of the deadliest malignancies, with a five-year survival rate below 13%. Although carbohydrate antigen 19-9 (CA19-9) is widely used as a prognostic biomarker for PDA, the functional roles of CA19-9-modified proteins in driving tumor progression remain poorly understood. Fibulin-3 (Fbln3), an extracellular matrix glycoprotein, is upregulated in advanced PDA and has been identified as a CA19-9-modified protein. However, its precise molecular mechanisms in CA19-9-positive PDA have not been elucidated. To investigate the function of Fbln3 in CA19-9-expressing PDA, we utilized our unique CA19-9-inducible mouse and 3D organoid models. We found that Fbln3 plays as a driver of tumor growth *in vivo* and regulates multiple oncogenic signaling pathways, including EGFR, STAT3, NF-kB, and SMAD2. Both genetic knockdown (KD) and pharmacological inhibition of Fbln3 suppressed activation of all four pathways, highlighting its central role in PDA. In addition, we found that Fbln3 promotes the expression of IL1A and TGFB, cytokines critical for tumor microenvironment (TME) remodeling. Orthotopic tumors with Fbln3 knockdown exhibited reduced myofibroblastic and antigen-presenting cancer-associated fibroblast populations. Moreover, Fbln3 knockdown increased CD8+ T cell infiltration, suggesting a shift toward a less immunosuppressive TME. Notably, Fbln3 knockdown did not affect tumor growth in immunodeficient mice, supporting an immune-dependent mechanism of tumor progression. Altogether, our findings demonstrate that Fbln3 mediates tumor progression and immunosuppressive TME remodeling in CA19-9-positive PDA through regulation of IL1A and TGFB across multiple oncogenic pathways. These results highlight Fbln3 as a promising therapeutic target for improving outcomes in PDA.

Transferable topic modeling enables cross-atlas mining and reveals developmental cellular lineage persistence in cancer

Ashmitha Rajendran[1,2], Parthiv Haldipur[6], Sonali Arora[3], Saanvi Subramanian[4], Kaustubh Grama[5], Leyre Merino Galan[2], David Johnson[2], Kimberly A. Aldinger[6,12,13], Jay Shendure[7,8,9,10], Kathleen J Millen[6,12], John H. Gennari[1], and Siobhan S. Pattwell[2,3,12]
[1]Department of Biomedical Informatics and Medical Education, University of Washington School of Medicine, Seattle, WA
[2]Ben Towne Center for Childhood Cancer and Blood Disorders Research, Seattle Children's Research Institute, Seattle, WA
[3]Human Biology Division, Fred Hutchinson Cancer Center, Seattle, WA
[4]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA
[5]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA
[6]Norcliffe Foundation Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, USA
[7]Department of Genome Sciences, University of Washington, Seattle, WA, USA
[8]Brotman Baty Institute for Precision Medicine, Seattle, WA, USA
[9]Seattle Hub for Synthetic Biology, Seattle, WA, USA
[10]Howard Hughes Medical Institute, Seattle, WA, USA
[12]Department of Pediatrics, University of Washington, Seattle, WA, USA
[13]Department of Neurology, University of Washington, Seattle, WA, USA

Single-cell transcriptomics transformed our understanding of cellular heterogeneity, yet cross-dataset comparison remains fundamentally limited by batch effects, technology differences, and inconsistent annotation frameworks. These challenges have impeded efforts to connect developmental programs with disease states which creates a critical gap for understanding pediatric cancers with suspected developmental origins. Here, we demonstrate that topic modeling, an unsupervised natural language processing technique, overcomes these barriers by learning transferable transcriptional genetic signatures that generalize across datasets, technologies, and biological contexts without requiring data integration. Applying this framework to over one million fetal cerebellar nuclei, we identify seven distinct topics that capture the developmental spectrum of rhombic lip progenitors through external granule layer (EGL) differentiation, including transitional states missed by conventional clustering. These topics transfer successfully across sequencing technologies (SPLiT-seq to sci-RNA-seq3), developmental timepoints, species (human to mouse), and from single-cell to bulk RNA sequencing of 876 medulloblastoma tumors. We reveal that Sonic hedgehog (SHH) medulloblastoma subtypes retain these topics, corresponding to distinct stages of EGL development. Our findings highlight that developmental timing at tumor initiation fundamentally shapes tumor biology, with immediate implications for subtype-specific therapeutic strategies in pediatric brain cancer. Our workflow establishes topic modeling as a scalable solution for mining expanding genomic atlases with broad applications across different datasets, technologies, and biological contexts.

PIKfyve supports a multicellular metabolic program maintaining glycosphingolipid homeostasis within the PDAC tumor microenvironment

Anna Trimble[1,2], Casie Kubota[2,3], Elaine Zhao[2,4], Maureen Ruchhoeft[2], Jonathan Weitz[5], Woncheol Jung[6], Kristina Peck[3], Satoshi Ogawa[3], Tae Gyu Oh[6], Andrew Lowy[5], Dannielle Engle[3,4], and Christian Metallo[1,2]

[1]Department of Bioengineering, University of California San Diego, La Jolla, CA, 92093, USA

[2]Department of Molecular and Cell Biology, Salk Institute for Biological Studies, La Jolla, CA, 92037, USA

[3]Department of Regulatory Biology, Salk Institute for Biological Studies, La Jolla, CA, 92037, USA

[4]Department of Biological Sciences, University of California San Diego, La Jolla, CA, 92093, USA

[5]Department of Surgery, Moores Cancer Center, University of California San Diego, La Jolla, CA, 92037, USA

[6]Department of Oncology Science, Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, 73104, USA

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy for which effective therapeutics are limited. Metabolic dysregulation within these tumors can support cancer cell survival within the austere tumor microenvironment (TME) and facilitate therapeutic resistance. The PDAC TME is also characteristically diverse, with abundant tumor and stromal cells contributing to tumor metabolism. By applying metabolic flux analysis to multiple models of PDAC, we determined that the TME significantly influences lipid metabolism within these tumors. While 2D cell cultures typically rely on *de novo* fatty acid synthesis to support complex lipid synthesis, PDAC cells within the native TME rely on salvage pathways supported by the high local fatty acid availability. Glycosphingolipids (GSLs), a diverse class of biologically active membrane lipids with critical functions promoting tumor proliferation and immune evasion, are highly synthesized within the PDAC tumor microenvironment through the glycosylation of salvaged lipids. While glycosphingolipid synthesis is not broadly sensitive to lysosomal inhibitors like chloroquine, the inhibition of the lipid kinase PIKfyve, a known effector of lysosomal function, significantly perturbs the glycan and lipid metabolism supporting GSL homeostasis. Using single cell RNASeq and LC-MS/MS lipidomics, we determine that the GSL profile of PDAC tumors is, itself, indicative of the cellular diversity of the TME. Tumor cells and stromal cells contribute distinct glycosphingolipid classes, with tumor cells expressing diverse gangliosides while fibroblasts produce abundant globosides. Interestingly, gangliosides and globosides respond to PIKfyve inhibition differently, with tumor cell-intrinsic GM3 synthesis being particularly sensitive. Together, this demonstrates that tumor cells and fibroblasts have distinct glycosphingolipid metabolism and that ganglioside synthesis may be a targetable vulnerability for PDAC tumor cells within the TME.

Lineage plasticity programs link mammary gland regeneration with breast cancer-associated states

Queralt Vallmajo-Martin[1,2], Nikki K. Lytle[3], Zhibo Ma[2], Sumana Srinivasan[4], Divya Murali[4], Christopher Dravis[2], Kavitha Mukund[4], Shankar Subramaniam[4], Deepshika Ramanan[1], and Geoffrey M. Wahl[2]
[1]NOMIS Center for Immunobiology and Microbial Pathogenesis
[2]Gene Expression Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA
[3]Department of Surgery, Medical College of Wisconsin, Milwaukee, WI, USA
[4]Department of Bioengineering, University of California, San Diego, La Jolla, CA, USA

The most aggressive cancers, such as triple negative basal-like breast cancer (TNBC), have high intratumoral heterogeneity that is often attributed to cell state reprogramming. This plasticity contributes to cancer progression, treatment resistance, and ultimately cancer death. Yet, the relevant mechanisms that enable cell state reprogramming in the mammary gland remain underexplored. To address this, we generated a dual-color CRISPR mouse model to distinguish basal (Krt14-mClover) from luminal (Krt18-tdTomato) cells, enabling real-time tracking of state transitions. In a transplantation paradigm, we found that transplanted basal cells rapidly exhibited a hybrid basal-luminal cell state, resembling embryonic mammary progenitors expressing Sox11, a tightly regulated differentiation factor typically shut off before birth. Notably, Sox11 knockout basal cells were unable to reconstitute the mammary gland, indicating that embryonic-like reprogramming is necessary for basal cells to regenerate the mammary gland. Further, we found Sox11 expressed across murine TNBC models, untreated BRCA1-mutant human TNBCs, and patient ductal carcinoma *in situ* (DCIS) and advanced TNBC samples, validated through immunostainings. Importantly, knocking out SOX11 in the MDA468 human TNBC cell line significantly delayed tumor growth in orthotopic transplantation models, suggesting a functional role for SOX11 in tumor progression. These findings imply that Sox11-expressing cells might be developmentally plastic intermediates in TNBC malignant progression. Ongoing studies aim to explore how these plastic cells interact with their microenvironment, particularly immune cells. Collectively, this work highlights shared lineage plasticity programs in mammary regeneration and early breast tumorigenesis, and reveals actionable therapeutic strategies to mitigate tumor heterogeneity and halt tumor progression.

Base-editor–based RNA recording reveals an mTOR-dependent mechanism linking IGF2BP3 RNA binding to translational control in cancer

Nadav Wallis, Will Brotheres, Benn Cravatt, and Gene Yeo
Cellular and Molecular Medicine, University of California San Diego

RNA-binding proteins (RBPs) play central roles in post-transcriptional gene regulation, yet methods to quantitatively measure RNA–protein interactions in living cells and link them to functional outcomes remain limited. Here we employ STAMP, a base-editor–based RNA recording approach in which RBPs are fused to a APOBEC1 to generate transcriptome-wide C-to-U edits at sites of RNA interaction. This strategy enables quantitative measurement of RNA binding at the transcript level in living cells without reliance on immunoprecipitation-based approaches.

Using STAMP, we investigated the oncogenic RNA-binding protein IGF2BP3, which is re-expressed in multiple cancers and promotes malignant growth. Activity-based protein profiling (ABPP) using stereochemically defined libraries of covalent compounds, performed in collaboration with the Cravatt laboratory, identified a small molecule as a covalent ligand of IGF2BP3. In living cells, STAMP measurements revealed widespread reductions in RNA engagement following treatment that depend on covalent modification of cysteine 194, demonstrating direct compound engagement and functional inhibition of IGF2BP3.

Mechanistically, mutational analysis identified a regulatory hotspot at the interface between the intrinsically disordered region (IDR) and the KH1 RNA-binding domain of IGF2BP3, encompassing Cys194 and nearby mTORC2-dependent phosphorylation sites. Phosphorylation at this interface promotes RNA binding, while inhibition of mTOR signaling reduces IGF2BP3 engagement with newly synthesized transcripts. These findings position the IDR–KH1 boundary as a regulatory hub linking IGF2BP3 RNA-binding activity to mTOR-dependent signaling pathways that coordinate cellular growth and metabolic demand.

Transcriptome-wide target analysis revealed that IGF2BP3 preferentially associates with TOP-enriched transcripts encoding core components of the translational machinery, including ribosomal proteins and translation factors. Disruption of IGF2BP3 binding reduces global translational output without substantially altering steady-state RNA abundance, indicating that IGF2BP3 primarily regulates translational machinery gene expression, at the level of translation.

Together, these findings uncover a feed-forward regulatory circuit in which mTOR-dependent phosphorylation promotes IGF2BP3 binding to translation-related transcripts, reinforcing protein synthesis programs that support tumor growth and metabolic adaptation.

PIN1 prolyl isomerase plays a crucial role in regulating the crosstalk between bladder cancer cells and fibroblasts

Xue Wang and Tony Hunter
Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037 USA

Urinary bladder cancer (BLCA) poses a serious public health challenge and is the most common cancer in the urinary system, with ~84K estimated new cases (76.5% men), and ~13K male/~5K female deaths in the US in 2026. Pin1 (protein interacting with never in mitosis A-1) is a phospho-dependent peptidyl prolyl cis/trans isomerase (PPIase), consisting of an N-terminal pSer/Thr.Pro-binding WW domain, linked to a C-terminal catalytic PPIase domain. Previous work shows that PIN1 participates in both initiation and progression of human cancer types. Moreover, recent studies have highlighted the relevance of the tumor microenvironment (TME) in cancer biology with translational implications. Cancer-associated fibroblasts (CAFs) are a prominent, heterogeneous population of cells in the TME. Moreover, our recent work has demonstrated a negative enrichment of SREBP2-driven cholesterol metabolism pathways and a decrease in free/total cholesterol levels in PIN1-knockout BLCA cells. However, functional roles of PIN1 in bladder fibroblasts or in the crosstalk between tumor cells and fibroblasts and the underlying molecular mechanisms remain largely unknown.

Here we find that both PIN1 knockout human normal fibroblasts (NFs) and CAFs are less proliferative compared to control cells. PIN1 knockout BLCA cells co-cultured with fibroblasts remarkably facilitate cell proliferation compared to PIN1 knockout BLCA cells only *in vitro*. Apart from the 2D culture system, we utilize human BLCA cells, T24 and 5637-formed organoids as predictors of therapy response and discover that fewer organoids are formed and less cell viability are shown when treated with Pin1's inhibitors/simvastatin (HMG-CoA Reductase's inhibitor) combination, compared to no inhibitor or single inhibitor treatment *in vitro* and significant reduction in tumor burden when treated with inhibitors *in vivo*. Similarly, we discover that Pin1 enhances mouse fibroblasts proliferation and Pin1-deleted fibroblasts significantly increase the organoid-formation capacity of mouse BLCA cells, MB49. Moreover, mouse NFs and TGFβ-treated myo-fibroblasts promote MB49 cells-formed organoids to be invasive. Further co-culture assays give hints that Notch, SREBP2-mediated cholesterol biosynthesis and STAT3 pathways may involve in the regulation of PIN1 in the crosstalk between tumor cells and fibroblasts. Together, these findings emphasize that PIN1 can act as a driver and potential therapeutic target in bladder cancer.

Dissecting the epigenetic inter- and intra-tumor heterogeneity of glioma at single-cell resolution

Yue Wu[1,4], King Hung[8], Wubin Ding[1], Laura Kellman[1], Jingtian Zhou[2,3], Kaushik Komandur[1], Zhiyi Deng[1], Rosa G Castanon[1], Anna Bartlett[1], Joseph R. Nery[1], Ethan Ashley[4,9], Christian Metallo[9], Jesse R. Dixon[5*], Thomas Beaumont[6*], and Joseph R. Ecker[1,7*]

[1]Genomic Analysis Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA
[2]Arc Institute, Palo Alto, CA, USA
[3]Bioinformatics and Systems Biology Program, University of California San Diego, La Jolla, CA, USA
[4]Division of Biological Sciences, University of California San Diego, La Jolla, CA, USA
[5]Peptide Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA. Stanford, CA, USA
[6]Department of Neurological Surgery, University of California San Diego, School of Medicine, La Jolla, CA, USA
[7]Howard Hughes Medical Institute, Salk Institute for Biological Studies, La Jolla, CA, USA
[8]Department of Neuroscience, Scripps Research, La Jolla, CA, USA
[9]Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA

Adult diffuse gliomas include IDH-mutant astrocytomas and oligodendrogliomas, as well as IDH-wildtype glioblastomas (GBM), yet how genetic and epigenetic programs coordinate cancer progression within individual tumors remain poorly understood. Here we apply joint single-nucleus methylome and 3D genome profiling (snm3C-seq) to 72,299 cells from 43 primary gliomas, simultaneously resolving DNA methylation and chromatin conformation states at single-cell resolution. We found that tumor-associated normal cells harbor copy number alterations and focal methylation changes, reflecting early neoplastic progression. Malignant cells predominantly occupy astrocyte-like (AC-like) and oligodendrocyte precursor cell-like (OPC-like) states. Within these malignant populations, joint profiling reveals that chromatin architectural reorganization precedes DNA methylation remodeling during cell-state transitions, and the transition is multidirectional in IDH wild-type glioblastoma. In IDH-mutant tumors, hypermethylation disrupts CTCF-mediated chromatin loops in a motif- and context-specific manner, redirecting regulatory contacts at cancer-relevant loci. In IDH-WT glioblastomas, certain forms of extrachromosomal DNA, such as the EGFRvIII variant, creates distinct genome-wide methylation profiles, whereas structural variants constitute a largely orthogonal axis of intratumoral heterogeneity. Together, these findings reveal that glioma evolution is collectively regulated by genetic and epigenetic programs that can either evolve in pairs or in parallel.

10010 N. Torrey Pines Rd.
La Jolla, CA 92037-1099
(858) 453-4100
www.salk.edu