T32 Cancer Symposium

Friday, February 10, 2023
Salk Institute for Biological Studies
La Jolla, California

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T32 Cancer Symposium Agenda

Friday, February 10, 2023

8:30 AM **REGISTRATION AND BREAKFAST**
9:30 AM **Welcoming Remarks**

Session I

9:35 AM Speaker Introduction
9:40 AM **Liling Wan, UPenn**
Chromatin regulator in cancer: new mechanisms and therapeutic opportunities
10:20 AM Speaker Introduction
10:25 AM **Ron Evans, Salk Institute**
Colon cancer: bench to bedside

11:05 AM **MORNING BREAK**

Session II

11:15 AM Selected Short Talk: **Brittany Jewell, Salk Institute**
Decoding the role of AMPK in colorectal cancer
11:30 AM Selected Short Talk: **Chiung-Ying Chang, Pfizer**
First-in-class KAT6A/KAT6B inhibitor CTx-648 (PF-9363) demonstrates potent anti-tumor activity in ER+ breast cancer with KAT6A dysregulation
11:45 AM Speaker Introduction
11:50 AM **Gordon Mills, OHSU**
Targeting adaptive responses in cancer through SMMART clinical trials

12:30 PM **LUNCH**

Session III

1:10 PM Speaker Introduction
1:15 PM **Darrell Irvine, MIT**
Engineering the stimulation of CAR T cells in lymphoid organs and tumors to eradicate solid tumors
1:55 PM Selected Short Talk**: Jan Lumibao, Salk Institute**
Developing and utilizing patient-derived organoid models to study pancreatic disease
2:10 PM Selected Short Talk: **Corina Antal, Salk Institute**
A super-enhancer regulated RNA-binding protein cascade drives pancreatic cancer
2:25 PM **Pop Talks**
Valentin Cracan, The Scripps Research Institute
A genetically encoded tool to increase cellular NADH/NAD+ ratio in living cells
Thomas Mann, Salk Institute
A molecular clock that controls CD8+ T cell function and exhaustion
Takuya Nakagawa, UCSD
A novel new treatment strategy for HPV-associated oropharyngeal cancer via human viral hybrid extrachromosomal DNA
2:35 PM Speaker Introduction
2:40 PM **Angela Koehler, MIT**
Attenuating Oncogenic Transcription
3:20 PM **Thanks and Wrap Up**

3:30 PM **RECEPTION AND POSTER SESSION**

Selected Short Talks

First-in-class KAT6A/KAT6B Inhibitor CTx-648 (PF-9363) Demonstrates Potent Anti-tumor Activity in ER+ Breast Cancer with KAT6A Dysregulation

Shikhar Sharma[1], Chiung-Ying Chang[1], Jay Chung[1], Sean Uryu[1], Amanda Rickard[1], Natalie Nady[1], Showkhin Khan[1], Zhenxiong Wang[1], Yong Zhang[1], Haikuo Zhang[1], Pei-Pei Kung[1], Eric Greenwald[1], Karen Maegley[1], Patrick Bingham[1], Hieu Lam[1], Ylva E. Bozikis[2,3], Hendrik Falk[2,4,5], Elizabeth Allan[2,4], Vicky M. Avery[2,7], Miriam S. Butler[2,8], Michelle A. Camerino[2,3], Catalina Carrasco-Pozo[2,7], Susan A. Charman[2,3], Melissa J. Davis[2,4,5], Mark A. Dawson[5,8], Sarah-Jane Dawson[5,8], Melanie de Silva[2,4], Matthew L. Dennis[2,6], Olan Dolezal[2,6], Rachel Lagiakos[2,3], Geoffrey J. Lindeman[4,5], Laura MacPherson[5,8], Stewart Nuttall[2,6], Thomas S. Peat[2,6], Bin Ren[2,6], Alexandra E. Stupple[2, 3, 9], Elliot Surgenor[4], Chin Wee Tan[4,5], Tim Thomas[4, 5], Jane E. Visvader[4, 5], Anne K. Voss[4,5], Francois Vaillant[4,5], Karen L. White[2, 3], James Whittle[4, 5], Yuqing Yang[4, 5], Soroor Hediyeh-Zadeh[4, 5], Paul A. Stupple[2, 3,9,] Ian P. Street[2,4,5,10,11,12], Brendon J. Monahan[2,4,5,9], and Thomas Paul[1]

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KAT6A is a lysine histone acetyltransferase (HAT) of the MYST family of HATs. KAT6A, and its paralog KAT6B, have been shown to acetylate histone H3K23Ac and regulate diverse biological processes, including transcription, cell-cycle progression, stem cell maintenance and development. Molecular dysregulation of KAT6A has been observed in several cancers, including amplifications in breast, lung, ovarian cancer along with oncogenic fusions in AML. In breast cancer, KAT6A is amplified as part of the 8p11 amplicon in 10-15% of the patient population, which correlates with a worse clinical outcome in the estrogen receptor+ (ER+) subtype. Here we present identification of a first-in-class potent KAT6A/KAT6B tool inhibitor CTx-648 (PF-9363), that possesses high selectivity versus other MYST family members (KAT7, KAT5, KAT8) and other KATs, demonstrating anti-tumor activity in breast cancer. Using genetic and pharmacological approaches, we have demonstrated several ER+ breast cancer cell lines including KAT6A amplified and over-expressing models, are dependent on KAT6A enzymatic function. Epigenomic profiling studies using bulk and nascent RNA-seq combined with ATAC-seq revealed CTx-648 leads to downregulation of a specific set of genes involved in ESR1 pathway, cell cycle and stem cell pathways. *In vivo* target validation studies showed strong anti-tumor activity of CTx-648 in several ER+ breast cancer cell line and patient-derived xenograft models, including models harboring endocrine therapy resistance ESR1 mutations, highlighting promise for this novel therapy in ER+ breast cancer population. Based on the strength of the pre-clinical data, a selective KAT6 inhibitor (PF-07248144) is now commencing a Phase 1 clinical study in Advanced or Metastatic Solid Tumors.

A Super-Enhancer Regulated RNA-Binding Protein Cascade Drives Pancreatic Cancer

Corina E. Antal, Corina E. Antal[1], Tae Gyu Oh[1], En-Ching Luo[2], Brian Yee[2], Tania Campos[3], Hervé Tiriac[4,5], Katherine Rothamel[2], Zhang Cheng[6], Henry Jiao[6], Allen Wang[6], Nasun Hah[1], Elizabeth Lenkiewicz[7], Morgan L. Truitt[1], Gabriela Estepa[1], Ester Banayo[1], Senada Bashi[1], Edgar Esparza[4,5], Ruben Munoz[8], Jolene K. Diedrich[9], Nicole M. Sodir[3,10], Jasmine R. Mueller[2], Erkut Borazanci[8,11], David Propper[11], Daniel Von Hoff[8,11], Christopher Liddle[12], Ruth T. Yu[1], Annette R. Atkins[1], Haiyong Han[9], Andrew M. Lowy[4,5], Michael T. Barrett[7], Gerard I. Evan[3], Gene W. Yeo[2], Michael Downes[1], and Ronald M. Evans[1]

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Pancreatic ductal adenocarcinoma (PDAC) has the worst 5-year survival rate of all major cancers and is thus in dire need of new therapeutic options. While the common mutational landscape and drivers of PDAC have been identified, this knowledge has yet to translate into durable treatments as these targets have proven intractable. Consequently, we set out to identify novel epigenetic vulnerabilities in the molecular pathways required to sustain tumor growth through unbiased identification of super-enhancers (SEs), which are genomic regions with high transcription factor binding densities and active histone marks that function as regulatory nodes at the transcriptional level to establish cell identity and behavior. Our work uncovered and validated several potential pancreatic cancer targets, both *in vitro* and *in vivo*. Among these targets we identified a druggable SE-mediated RNA-binding protein (RBP) cascade that supports PDAC growth through enhanced mRNA translation. This cascade is driven by a SE associated with heterogeneous nuclear ribonucleoprotein F (*HNRNPF*) that controls the levels of this RBP. We show that hnRNP F regulates the expression of protein arginine methyltransferase 1 (PRMT1) by binding to and stabilizing its mRNA. Using quantitative mass spectroscopy, we identify the translational mediator ubiquitin-associated protein 2-like (UBAP2L) as a downstream target of PRMT1 that mediates its effect on protein synthesis rates. We show that loss of methylation within UBAP2L's RNA-binding domain decrease its affinity for RNA, thus impacting ribosome biogenesis and translation. Using orthotopic mouse models of PDAC, we demonstrate that all three of these genes and the regulatory SE are essential for PDAC growth and coordinately regulated by the Myc oncoprotein. Additionally, we show that these genes are also upregulated in human PDAC and that the *HNRNPF* SE is present in biopsies from PDAC patients. Importantly, we demonstrate that modulation of this RBP network by small molecule PRMT1 inhibitors reveals a unique vulnerability in Myc-high pancreatic cancer patient organoids and markedly reduces tumor growth *in vivo*. Together, this work highlights a functional link between epigenetic regulation and mRNA translation and identifies components that comprise unexpected therapeutic targets for PDAC.

Pop Talks

A Genetically Encoded Tool to Increase Cellular NADH/NAD+ Ratio in Living Cells

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Impaired reduction/oxidation (redox) metabolism is a key contributor to the etiology of many diseases, including primary mitochondrial disorders, neurodegeneration, and cancer. However, mechanistic studies of redox imbalance remain challenging due to limited strategies which can perturb cellular redox metabolism and model pathology in various cellular, tissue, or organismal backgrounds without creating additional and potentially confounding metabolic perturbations. To date, most studies involving impaired redox metabolism have focused on oxidative stress and reactive oxygen species (ROS) production; consequently, less is known about the settings where there is an overabundance of reducing equivalents, termed reductive stress. NADH reductive stress has been modeled using pharmacologic inhibition of the electron transport chain (ETC) and ethanol supplementation. Still, both these methods have significant drawbacks. Here, we introduce a soluble transhydrogenase from E. coli (EcSTH) as a novel genetically encoded tool to promote NADH overproduction in living cells. When expressed in mammalian cells, EcSTH, and a mitochondrially-targeted version (mitoEcSTH), can elevate the NADH/NAD+ ratio in a compartment-specific manner. Using this tool, we determine the metabolic and transcriptomic signatures of NADH reductive stress in a panel of cancer cell lines. We also find that cellular responses to NADH reductive stress, including blunted proliferation, are cell lines specific and identify the metabolic reactions that sense changes in the cellular NADH/NAD+ balance. Collectively, our novel genetically encoded tool represents an orthogonal strategy to perturb redox metabolism and explore unique aspects of metabolic remodeling in cancer.

A Molecular Clock that Controls CD8+ T Cell Function and Exhaustion

Thomas Mann, Shixin Ma, Anna-Maria Globig, Jesse Furgiuele, Hokyung K. Chung, Bryan McDonald, and Susan M. Kaech
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During cancer and chronic viral infections, the persistence of antigen progressively causes CD8+ T cells to differentiate into a dysfunctional PD1+ "exhausted" state, with reduced production of inflammatory cytokines relative to effector cells that form during acute infections. Antigen and costimulation signals activate kinase cascades to induce distinct T cell transcription programs, but how T cells distinguish acute and chronic signals to program the exhausted or effector transcriptional states remains poorly understood. We found that members of the protein kinase C (PKC) family function together as a "molecular clock," sensing acute or chronic agonism to drive distinct transcriptional programs. Continuous stimulation of PKC induces many features of T cell exhaustion, including a loss of production of the cytokines IFNγ and TNF, upregulation of inhibitory receptors and TOX, and altered expression of the proteins in the AP-1 family. Mechanistically, CD8+ T cells express several different PKC proteins, and chronic agonism of PKC leads to degradation of multiple family members and selective maintenance of only one PKC protein, PKC-η. This "PKC switch" alters downstream signaling to support the transcriptional reprogramming of T cells into a terminally exhausted state. In summary, continuous signaling through PKCs causes changes in the output from these kinases initially at the protein level, driving transcriptional changes downstream of PKC targets in the AP-1 transcription factor family and thus allowing further widespread transcriptional and functional changes that characterize T cell exhaustion.

A Novel New Treatment Strategy for HPV-Associated Oropharyngeal Cancer via Human-Viral Hybrid Extrachromosomal DNA

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Human Papillomavirus-associated Oropharyngeal Cancer (HPVOPC) is dramatically increasing over the last two decades and became the 2nd-fastest growing cause of solid organ cancer death in the U.S. However, the molecular mechanism underlying this phenomenon remains elusive. Recently we identified Human-viral hybrid ecDNA (hybrid ecDNA), a fusion of the human genome and HPV genome generated from the integration site, in HPVOPC and this structure has potential to build an innovative treatment strategy in HPVOPC.

To identify the hybrid ecDNA, whole genome sequencing (WGS) and RNA-seq were performed on HPVOPC cell lines and Patient derived xenograft (PDX) tumors from clinical tumor samples of HPVOPC patients. Based on WGS and RNA-seq, Amplicon Architect (AA) and Viral Integration and Fusion Identification (ViFi) were used to detect Viral integration and hybrid ecDNA. For the anatomical validation of hybrid ecDNA, multi-FISH targeting human genome and HPV genome on hybrid ecDNA were performed using metaphase spread HPVOPC cell line and short time cultured PDX tumor cells. To elucidate the intra hybrid ecDNA interaction, we performed ChIP-seq using cell lines and PDX tumor for H3K27ac, H3K4me1, and H3K4me3. To further examine the involvement of hybrid ecDNA in cancer growth, we performed a proliferation assay using CRISPR interference (CRISPRi) or BET inhibiter; a therapeutic agent that targets hybrid ecDNA, using each of hybrid ecDNA(+/-) cell lines. BET inhibiter treatment was also performed using PDX tumor model.

In both of cell lines and PDX tumors, we identified hybrid ecDNA(+) tumors. In multi-FISH for the ecDNA specific and HPV probe, we identified overlapped signaling in outside of the chromosome in metaphase spread cells, confirming the existence of hybrid ecDNA. ChIP-seq of ecDNA(+) cell line showed that the hybrid ecDNA sequence contained an active enhancer. Strikingly, this active enhancer was not exist in the hybrid ecDNA(-) cell line in the same region, suggesting this enhancer was newly created after the HPV integration to this region. To check the functional interaction of this active enhancer, we used CRISPRi to inactivate this enhancer targeted by sgRNA. Compare to nontarget control, inactivating this enhancer significantly inhibited the proliferation only in hybrid ecDNA(+) cell line (P=0.006). In addition, BET inhibitor treatment targeting to hybrid ecDNA(+) tumor showed the significant inhibition of tumor growth in both of cell lines and PDX tumor, suggesting a possibility of a new targeted therapy.

Hybrid ecDNA contains active enhancer that is never included in the hybrid ecDNA(-) tumor. Targeting to this enhancer (CRISPRi) or hybrid ecDNA (BET inhibitor) showed significant inhibition of tumor growth in hybrid ecDNA(+) cell lines and PDX that is not shown in hybrid ecDNA(-) tumor. These results suggested the new innovative treatment strategy for hybrid ecDNA(+) HPV.

Posters

Poster Sessions

Nasiha S. Ahmed, Jovylyn Gatchalian, Josephine Ho, Mannix J. Burns, Nasun Hah, Zong Wei, Michael Downes, Ronald M. Evans, and Diana C. Hargreaves

Salk Institute for Biological Studies

BRD9 Regulates Interferon-Stimulated Genes During Macrophage Activation via Cooperation with BET Protein BRD4

Joshua Baumgart, Anwesh Kamireddy, Jolene Diedrich, John Asara and Reuben J. Shaw

Salk Institute for Biological Studies

Rasal2 as a SIK1/3 Substrate that Mediates Tumor Suppressor Function of LKB1 in Lung Adenocarcinoma

Olga M. Chaim, Shunichiro Miki, Briana C. Prager, Anthony Y. Cheong, Nancy K. Tran, Jacqueline Lara, Melissa S. Barlow, J. Silvio Gutkind, Jeremy N. Rich, Shigeki Miyamoto, Frank Furnari & Joan Heller Brown

University of California San Diego

Gα12 Signaling Maintains Glioblastoma Stem Cells and Tumor Invasion

Christophe Le Clorennec, Divya Subramonian, Yuchen Huo, and Peter E. Zage

University of California San Diego

UBE4B Interacts with the ITCH E3 Ubiquitin Ligase to Induce Ku70 and c-FLIPL K63/K48 Branched Polyubiquitination Leading to Ku70 and c-FLIPL Proteasomal Degradation and Enhanced Neuroblastoma HDACi-mediated Caspase 8 Dependent Apoptosis

Suzanne Dufresne, Louis Parham, Anvita Komarla, Kristiana Wong, and Christina Towers

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Targeting Autophagy in PDAC

Mehrak Javadi-Paydar, Cristina Salmerón, Kimberly Q. Pham, Jane Smitham, and Paul A. Insel

University of California San Diego

Age-Dependent, Parallel Increases in GPCR and Collagen Expression and Fibrosis in Pancreatic Tumors of KPC Mice

Melissa A. Johnson, Siva Karthik Varanasi, Kailash Chandra Mangalhara, Sagnika Ghosh, Dan Chen, Alexandra G. Moyzis, Susan M. Kaech, and Gerald S. Shadel

Salk Institute for Biological Studies

Mitochondrial Regulation of Tumor Interferon Response

Corey Jones-Weinert and Jan Karlseder

Salk Institute for Biological Studies

Understanding the Role of ZBP1 Filaments in the Prevention of Age-Associated Cancer Initiation

Anvita Komarla, Celina Shen, Suzanne Dufresne, Payel Mondal, Sienna Rocha, Louis Parham, and Christina Towers

Salk Institute for Biological Studies

Identifying Unique Pathway Dependencies and Novel Mechanisms of Quality Control in Autophagy-Deficient Cancer Cells

Xiaoxue Lin, Alexandra Fowler, Chelsea Bottomley, Shira Okhovat, Kristina Peck, and Dannielle Engle

University of California San Diego

Smoking Induces the Redox Pathway to Facilitate Pancreatic Ductal Adenocarcinoma (PDA) Initiation and Progression

Matthew B. Maxwell, Marianne S. Hom-Tedla, Jawoon Yi, Mannix J. Burns, Jingting Yu, Helen M. McRae, Katherine E. Coakley, Josephine Ho, Ramez N. Eskander, Emily C. Dykhuizen, Gerald S. Shadel, Susan M. Kaech, and Diana C. Hargreaves

Salk Institute for Biological Studies

Loss of the ARID1A Tumor Suppressor Activates an R-Loop Driven STING-Type I Interferon Signaling Axis that Promotes Anti-Tumor Immunity

Payel Mondal
Salk Institute for Biological Studies
Metabolic Biomarkers for Circumventing Autophagy Inhibition in Cancer Cells

Alexandra Moyzis, Melissa A. Johnson, Matthew P. Donnelly, and Gerald S. Shadel
Salk Institute for Biological Studies
Targeting Mitochondrial Biogenesis and Antioxidant Pathways to Prevent T Cell Exhaustion

Christopher W. Murray, Antonio F.M. Pinto, Hazel Shan, Dan Tan, Alan Saghatelian, and Reuben J. Shaw
Salk Institute for Biological Studies
Exploring the Functional Landscape of Lipid Metabolism in Non-Small Cell Lung Cancer

Louis Parham, Suzanne Dufresne, Anvita Komarla, and Christina Towers
Salk Institute for Biological Studies
Understanding the Molecular Mechanisms Underlying Acquired Resistance to Autophagy Inhibition in Pancreatic Cancer Cells

Mara Gilardi, Monika Ramos, Kendrick Nguyen, A-Reum Kim, Marco Proietto, and Daniel Hollern
Salk Institute for Biological Studies
New Mouse Model Tools to Discover Effective Immune Therapies for Triple Negative Breast Cancer Metastases

Tobias T. Schmidt, Marco R. Cosenza, Candy Haggblom, Kat Lande, April Williams, Jan O. Korbel, and Jan Karlseder
Salk Institute for Biological Studies
Evolution of Cells Progressing through Replicative Crisis to Immortality

Qiyuan Yang, Yuqiong Liang, and Ye Zheng
Salk Institute for Biological Studies
PPARd Restricts Intratumoral Treg Function Through Negatively Regulating the CIITA-MHCII Axis

Shira Yomtoubian, TJ Rymoff, Debbie Ross, and Reuben Shaw
Salk Institute for Biological Studies

Deciphering the Role of AMPK in Pancreatic Cancer

BRD9 Regulates Interferon-Stimulated Genes During Macrophage Activation via Cooperation with BET Protein BRD4

Nasiha S. Ahmed*[1], Jovylyn Gatchalian*[1], Josephine Ho[1], Mannix J. Burns[1], Nasun Hah[2], Zong Wei[3], Michael Downes[3], Ronald M. Evans[3], and Diana C. Hargreaves[1]
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Macrophages induce a number of inflammatory response genes in response to stimulation with microbial ligands. In response to endotoxin Lipid A, a gene-activation cascade of primary followed by secondary-response genes is induced. Epigenetic state is an important regulator of the kinetics, specificity, and mechanism of gene activation of these two classes. In particular, SWI/SNF chromatin-remodeling complexes are required for the induction of secondary-response genes, but not primary-response genes, which generally exhibit open chromatin. Here, we show that a recently discovered variant of the SWI/SNF complex, the noncanonical BAF complex (ncBAF), regulates secondary-response genes in the interferon (IFN) response pathway. Inhibition of bromodomain-containing protein 9 (BRD9), a subunit of the ncBAF complex, with BRD9 bromodomain inhibitors (BRD9i) or a degrader (dBRD9) led to reduction in a number of interferon-stimulated genes (ISGs) fol- lowing stimulation with endotoxin lipid A. BRD9-dependent genes overlapped highly with a subset of genes differentially regulated by BET protein inhibition with JQ1 following endotoxin stimula- tion. We find that the BET protein BRD4 is cobound with BRD9 in unstimulated macrophages and corecruited upon stimulation to ISG promoters along with STAT1, STAT2, and IRF9, components of the ISGF3 complex activated downstream of IFN-alpha receptor stimulation. In the presence of BRD9i or dBRD9, STAT1-, STAT2-, and IRF9-binding is reduced, in some cases with reduced binding of BRD4. These results demonstrate a specific role for BRD9 and the ncBAF complex in ISG activation and identify an activity for BRD9 inhibitors and degraders in dampening endotoxin- and IFN-dependent gene expression.

Rasal2 as a SIK1/3 Substrate that Mediates Tumor Suppressor Function of LKB1 in Lung Adenocarcinoma

Joshua Baumgart[1], Anwesh Kamireddy[1], Jolene Diedrich[2], John Asara[3] and Reuben J. Shaw[1]
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Liver kinase B1 (LKB1) is a tumor suppressor that is inactivated in numerous human cancers. LKB1 is a master kinase that directly phosphorylates and activates a group of fourteen kinases known as 5' adenosine monophosphate-activated protein kinase related (AMPKR) protein kinases. The downstream signaling of LKB1 that mediates its tumor suppressor role has not been fully characterized but has been shown to depend on LKB1's kinase activity, suggesting that one or several AMPKR pathways are involved in this process. Better understanding of the downstream signaling of the fourteen AMPKRs is vital to complete understanding of the tumor suppressive function of LKB1. To this end, a phosphoproteomic screen was performed on paired cell lines generated through CRISPR mediated loss of LKB1. This screen identified several proteins that undergo an LKB1-dependent increase in phosphorylation on specific sites that conform to the optimal AMPKR substrate peptide sequence, including multiple small GTPase activating proteins (GAPs). After protein characterization, Rasal2 was identified as the most interesting candidate due to its *in vivo* tumor suppressive potential. Upon further characterization Sik1 and Sik3 were identified as the upstream kinase responsible for phosphorylating Rasal2, but further investigation is needed to understand their tumor suppressive role in NSCLC models.

Gα12 Signaling Maintains Glioblastoma Stem Cells and Tumor Invasion

Olga M. Chaim[1,5], Shunichiro Miki[2], Briana C. Prager[3], Anthony Y. Cheong[1], Nancy K. Tran[1], Jacqueline Lara[1], Melissa S. Barlow[1], J. Silvio Gutkind[1,3], Jeremy N. Rich[3,4], Shigeki Miyamoto[1], Frank Furnari[2] & Joan Heller Brown[1]
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Analysis of TCGA data reveals that glioblastoma multiforme (GBM) is associated with upregulation of GNA12 (Gα12), encoding the alpha subunit of the heterotrimeric G-protein G12, concomitant with overexpression of multiple G-protein coupled receptors (GPCRs) that signal through Gα12. To investigate the biological functions of Gα12 in GBM we used shRNAs to knock down Gα12 in human glioma stem cells (GSCs). Loss of Gα12 significantly attenuated tumor cell stemness and the expression of canonical stem cell genes. Gα12 knockdown (KD) had no apparent effect on orthotopic tumor size or mouse survival but there were striking histopathological differences at the tumor border, indicative of diminished invasive properties. *In vitro* GSC invasion was inhibited by Gα12 KD and stimulated by chemogenetic activation of Gα12. RNA sequencing identified differentially expressed genes related to cell adhesion and migration in Gα12 KD tumors. Notably, cells and tumors with Gα12 KD also showed increased expression of proneural genes, suggesting that Gα12 signaling promotes a more aggressive mesenchymal GBM phenotype. Thrombospondin-1 (THBS1), a prognostic marker of aggressive mesenchymal GBM, was one of the genes most repressed by Gα12 KD and THBS1 and Gα12 mRNA levels are highly correlated in TCGA. Furthermore, we demonstrate that THBS1 is required for Gα12-mediated cell migration *in vitro*, and that tumors derived from THBS1 KD cells showed less invasion into the mouse brain. Our findings reveal that Gα12 signaling regulates transcriptional programs for stemness and invasiveness of glioma stem cells and represents a potential signaling node for therapeutic intervention.

UBE4B Interacts with the ITCH E3 Ubiquitin Ligase to Induce Ku70 and c-FLIPL K63/K48 Branched Polyubiquitination Leading to Ku70 and c-FLIPL Proteasomal Degradation and Enhanced Neuroblastoma HDACi-mediated Caspase 8 Dependent Apoptosis

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Expression of the UBE4B ubiquitin ligase is associated with neuroblastoma patient outcomes, but the functional roles of UBE4B in neuroblastoma pathogenesis are not known. We evaluated interactions of UBE4B with the E3 ubiquitin ligase ITCH/AIP4 and the effects of UBE4B expression on Ku70, c-FLIPL, and p53 ubiquitination and proteasomal degradation by co-immunoprecipitation and Western blots. We also evaluated the role of UBE4B in apoptosis induced by HDAC inhibition in our neuroblastoma cell lines model using Western blots. UBE4B was found in a complex with ITCH, with binding mediated by WW domains in the ITCH protein. ITCH activation led to ITCH-UBE4B E3-E4 ubiquitin ligase complex formation and recruitment of Ku70 and c-FLIPL via ITCH WW domains, followed by Ku70 and c-FLIPL Lys48/Lys63 branched polyubiquitination and proteasomal degradation. Histone deacetylase (HDAC) inhibition induced Ku70 and c-FLIPL acetylation, leading to release of c-FLIPL and Bax from Ku70, increased Ku70 and c-FLIPL Lys48/Lys63 branched polyubiquitination via the ITCH-UBE4B complex, and induction of apoptosis. UBE4B depletion in our neuroblastoma cell lines model led to reduced polyubiquitination and increased levels of Ku70 and c-FLIPL proteins leading to the massive reduction of apoptosis induced by HDAC inhibition via stabilization of Ku70 and c-FLIPL proteins allowing the inhibition of the full caspase 8 activation. Our results have identified novel interactions and novel targets for UBE4B ubiquitin ligase activity and a direct role of the ITCH-UBE4B complex in responses of neuroblastoma cells to HDAC inhibition, suggesting that the ITCH-UBE4B complex plays a critical role in responses of neuroblastoma to therapy and suggesting a potential mechanism underlying the association of UBE4B expression with neuroblastoma patient outcomes.

Targeting Autophagy in PDAC

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most devastating diseases. Recent reports suggest that autophagy inhibition might improve survival for patients with PDAC. But, given that initial studies have already observed significant inherent and acquired resistance, it is critical to identify combination therapies to improve efficacy of autophagy inhibition and intercept resistance. Moreover, animal models have revealed a dual role of autophagy in PDAC where it prevents early tumorigenesis but then promotes progression. However, these models have been unable to dissect this mechanistic switch. With a better understanding of these opposing roles of autophagy in PDAC, our work could identify ideal contexts and combination therapies that preserve the anti-tumor roles of autophagy while blocking the pro-tumor functions.

In this context, our goal is to (1) identify new mechanisms of resistance to autophagy inhibition in PDAC and (2) provide a better understanding of the anti- to pro-tumor switch of autophagy that occurs during tumorigenesis.

We generated murine PDAC cell lines resistant to genetic inhibition of autophagy (Atg7 knock-out) as well as to pharmacological blockade of autophagy by treating the cells with increasing doses of hydroxychloroquine (HCQ) or MRT68921 (MRT). Interestingly, the HCQ-resistant cells showed resistance to MRT, and MRT-resistant cells showed resistance to HCQ, suggesting that these cells are sharing similar resistance mechanisms. Strikingly however, the Atg7 KO cells were as sensitive to these drugs as their Atg7 wild type counterparts. We are planning to submit these cells to RNA sequencing to decipher the similitudes and differences between the cell lines and provide a deeper understanding of mechanisms underlying resistance to autophagy inhibition in PDAC. In addition, we are currently developing a 3D organoid system to model the different tumorigenesis stages of PDAC. By deleting autophagy in the early versus late stage of malignancy, we will better understand the dual role of autophagy in PDAC and identify in which context autophagy inhibition can be used as a tool to counteract tumor cell growth. Together, our findings will help design better therapeutic strategies for PDAC patients.

Age-Dependent, Parallel Increases in GPCR and Collagen Expression and Fibrosis in Pancreatic Tumors of KPC Mice

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Pancreatic ductal adenocarcinoma (PDAC), the most prevalent type of pancreatic cancer, continues to have high mortality and is in need of new therapies. PDAC typically is fibrotic (desmoplastic) with increased collagen deposition. Human PDAC tumors also have increased expression of numerous GPCRs. We assessed the time-course of expression of fibrosis/collagen and such GPCR genes in KPC mice, which spontaneously develop PDAC tumors.
We analyzed male and female KPC PDAC tumors and non-KPC mice pancreas controls at age 4-9 weeks. We quantified fibrotic tissue accumulation in PDAC tumors and the pancreas and used qPCR to assess the expression of collagen 1A1 and 6 GPCRs: GPR68, GPRC5A, HRH1, PAR1, PAR2, and P2Y2. Statistical differences between the KPC and non-KPC mice were determined by two-way ANOVA. We used Pearson correlation coefficient to assess tumor expression of collagen 1A1 and the GPCRs at different ages of the KPC mice. Results: Tumor fibrosis increased in an age-dependent manner in parallel with tumor weight from weeks 4 to 9. COL1A1 expression increased as early as week 4 vs. non-KPC controls ($p < 0.1$, $n = 6$). Expression of the F2RL1/PAR2 and GPRC5A genes significantly increased by week 4 in KPC mice compared to control mice ($p < 0.1$, $n = 6$; $p < 0.01$, $n = 6$). Expression of GPR68/OGR1, HRH1 and F2R/PAR1 increased by week 6 in the KPC mice ($p < 0.0001$; $p < 0.0001$; $p < 0.001$, $n = 6$). Expression of collagen 1A1 and each of the GPCR genes were positively correlated in tumors at weeks 4 and 7, respectively ($r = >0.92$, $p < 0.01$ for all 6 GPCRs). Expression of each GPCR increased in fibrotic tumors and was positively correlated with one another. GPRC5A, PAR1, PAR2, P2Y2 and HRH1 were expressed in cancer cells isolated from KPC tumors. Conclusions: Expression of collagen 1A1 and multiple GPCRs thus increases in parallel with fibrosis in KPC mice, suggesting that the increase in these GPCRs, and perhaps others, may be a consequence of, or mechanistically related, to the increase in collagen 1A and fibrosis in PDAC tumors. Multiple GPCRs are expressed on KPC (and on human) PDAC cancer cells. One or more of these GPCRs may contribute to tumor biology and be potential biomarkers and/or therapeutic targets for PDAC.

Mitochondrial Regulation of Tumor Interferon Response

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Inflammation plays a critical role in tumorigenesis, paradoxically tempering and promoting tumor development. Pro-inflammatory cytokines such as type I and type II interferons (IFNs) initially defend against cancer development by inducing tumor cell death, however, continuous stimulation results in the selection of tumor subclones that have acquired immunosuppressive properties to enable disease progression. This process, referred to as cancer immunoediting, has become central to our understanding of the relationship between malignancy and the immune system. In the background of immunoediting, tumor cells also undergo metabolic adaptations which promote their malignant phenotype. Metabolic rewiring and immune evasion are both cancer hallmarks, however, their interdependencies are not well understood. Recent work from our lab and others has shown that mitochondrial electron transport chain activity is required for one of the primary functions of IFN stimulation, which is the induction of major histocompatibility complex I (MHC-I) expression on the surface of tumor cells for CD8+ T cell recognition and killing. Thus, mitochondria are poised as central mediators of anti-tumor immunity through their role in IFN response. To directly interrogate the relationship between mitochondrial metabolism and tumor IFN signaling, we modeled chronic IFN stimulation *in vitro* using the Yale University Mouse Melanoma (YUMM1.7) cell line and profiled resultant changes in tumorgenicity and mitochondrial function. Melanoma cells that were chronically exposed to IFN *in vitro* not only showed increased tumor growth and metastasis when transferred *in vivo*, but also had an associated downregulation of oxidative phosphorylation (OXPHOS). In addition, we have performed a metabolism-targeted CRISPR knock-out screen in the same YUMM1.7 cell line which has revealed roles for fatty acid metabolism and reactive oxygen species (ROS) in addition to OXPHOS in regulating IFN response. Ongoing studies are aimed at delineating whether there is a common energetic requirement for IFN response, or whether a more intricate regulatory network exists between specific mitochondrial function or fuel usage and IFN signaling. Expanding our understanding of the metabolic pathways that control IFN responses may lead to new ways to target tumor metabolism to bolster anti-tumor immunity and increase the efficacy of immunotherapy.

Understanding the Role of ZBP1 Filaments in the Prevention of Age-Associated Cancer Initiation

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Age regulated proliferative boundaries of primary human cells act as tumor suppressive pathways by preventing cells from accumulating an unstable genome, from losing growth regulatory checkpoints, and from becoming immortal. Cellular responses to telomere shortening comprise one such anti-tumorigenesis boundary. Following excessive replication-associated telomere shortening, telomeres become deprotected and activate a DNA damage response, triggering p53/RB-dependent senescence. Loss of cell cycle checkpoints allows cells to bypass this replicative senescence barrier and resume cell division, despite harboring dysfunctional telomeres. Continued telomere erosion results in chromosome fusions and genome instability, a state known as crisis. We discovered that cytoplasmic DNA generated from chromosome breakage-fusion cycles during crisis is sensed by cGAS/STING, leading to the induction of interferon-stimulated genes (ISGs) and a non-canonical form of autophagy that results in cell death, thereby connecting telomere shortening-driven crisis with the innate immune response and autophagy.

Expanding on this breakthrough together with the Shadel laboratory, we recently found that a short isoform of Z-DNA-binding protein 1 (ZBP1), ZBP1(S), is induced by activated cGAS/STING. ZBP1(S) forms filaments on mitochondrial outer membranes, which overlap with Mitochondrial Antiviral Signaling protein (MAVS) in crisis cells, thereby activating an inflammatory pathway that culminates in autophagic cell death. Simultaneously, the telomeric RNA TERRA, which is transcribed from subtelomeric promoters, is induced at dysfunctional telomeres in crisis cells. Exogenous induction of TERRA and ZBP1(S) triggers autophagic cell death in young cells with protected telomeres, highlighting the synergy between the two arms of this bifurcated pathway in clearing precancerous cells with critically short telomeres. We predict that this entirely novel pathway plays a central role in preventing the emergence of neoplastic cells during human aging, thereby representing an attractive target for intervention.

I will investigate how TERRA activates ZBP1(S). Specifically, I hypothesize that interaction with TERRA leads to conformational changes in ZBP1(S), thereby allowing it to form filaments and localize to mitochondria and trigger cell death via autophagy. With this information, I will explore the possibility of manipulating the TERRA-ZBP1(S)-MAVS axis. I hope to identify the features of TERRA relevant to ZBP1(S) activation and develop pharmacological tools to mimic this activation in cancer cells that aberrantly express ZBP1(S).

Identifying Unique Pathway Dependencies and Novel Mechanisms of Quality Control in Autophagy-Deficient Cancer Cells

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The process of autophagy, the cellular recycling and quality control pathway, is crucial to cellular health. In established tumors it promotes cancer cell growth by supporting cellular stress tolerance. Autophagy inhibition *in vivo* also shows decreased tumor volume and prolonged survival indicating a dependence on this pathway. These studies have fueled clinical trials to target autophagy in several tumor types. Although clinical trials have shown promising results, a significant portion of patients do not respond, and some patients respond initially but begin to relapse while still on autophagy inhibition therapy. In support of this phenomenon, molecular studies by Towers et al. have shown that autophagy-dependent breast and lung cancer can acquire resistance to autophagy inhibition and grow at a similar rate compared to their parental cell line. Additionally, autophagy-deficient cells had normal mitochondrial function indicating that mitochondrial quality control still occurs in the absence of mitophagy, a specific form of autophagy that maintains mitochondrial health. Work by Towers et al. showed that autophagy-deficient cells upregulate Mitochondrial Derived Vesicles (MDVs) which are small vesicles that can bud off the mitochondria and directly traffic to the lysosome. This pathway is relatively understudied and there are few known regulators. My thesis project revolves around understanding how autophagy-deficient cells can continue to survive without this crucial cellular homeostasis pathway. Specifically, we will use genome-wide CRISPR screening to identify new pathway dependencies in these autophagy-deficient cell lines in a high-throughput manner. We will also use a fluorescent reporter of mitochondrial degradation in our CRISPR library-tagged cells to identify novel regulators of mitochondrial delivery to the lysosome. This research could illuminate ideal combinatorial therapeutics with autophagy inhibitors, identify novel mechanisms of global cellular and mitochondrial quality control, and reveal key mediators of the MDV pathway.

Smoking Induces the Redox Pathway to Facilitate Pancreatic Ductal Adenocarcinoma (PDA) Initiation and Progression

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Pancreatic ductal adenocarcinoma (PDAC) has a 5-year survival rate of only 10%. It is one of the deadliest cancers, and is expected to become the second leading cause of cancer death by 2025.

Tobacco is one of the main risk factors for PDAC. Incidence of PDAC increases approximately 2-fold among people who use tobacco products and it is estimated that 20-35% of PDAC cases are caused by smoking . Besides their well-known role in mutagenesis, tobacco products induce oxidative stress and inflammation, thereby promoting PDAC initiation and progression. However, the effector pathways activated following tobacco carcinogen exposure remain unclear. The tumor microenvironment plays a substantial role in PDAC initiation and treatment response. Therefore, the impact of tobacco-induced chances in reactive oxidative species (ROS)-induced oxidative stress on the recruitment and function of the immune and fibroblast stromal compartment in PDAC would benefit from systematic study.

This project aims to address the impact of carcinogens in pancreatic cancer etiology. We hypothesize that carcinogens in tobacco products increase the transformation potential in the pancreas and contribute to disease initiation and progression through both intrinsic and extrinsic mechanisms. Specifically, we will investigate two major subclasses of carcinogens, cadmium (Cd) and benzo(a)pyrene (BaP) in normal and premalignant mouse pancreatic ductal organoid cultures. Cadmium is the inorganic, non-essential metal, and BaP is a member of the polycyclic aromatic hydrocarbon (PAH) family. The two products are found in tobacco and e-cigarette aerosols. We will focus on the impact of acute and chronic carcinogen exposure on cell viability, ROS levels, and redox pathway in both the epithelial compartment and microenvironment. To investigate carcinogen-mediated remodeling of the microenvironment, we will use organoid co-culture of macrophages, organoids, fibroblasts (MOrF). Ultimately, these findings will be validated using human pancreatic organoids and tumor slice culture.

Collectively, this project contributes to the understanding of tobacco-related PDA pathogenesis in two aspects. First, we will establish a novel carcinogenesis model using an advanced 3D organoid culture system. Upon carcinogen treatment, pancreatic ductal organoids can recapitulate physiologically relevant aspect of ductal cells and PDA during disease initiation and progression. Second, this project will elucidate a deeper connection between xenobiotic metabolism and pancreatic cancer by examining the intrinsic and extrinsic mechanisms by which carcinogens contribute to transformation.

Loss of the ARID1A Tumor Suppressor Activates an R-Loop Driven STING-Type I Interferon Signaling Axis that Promotes Anti-Tumor Immunity

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Clinical trials have identified ARID1A mutations as enriched among patients who respond favorably to Immune Checkpoint Blockade (ICB) in a number of solid tumor types in a manner that is independent of microsatellite instability. We developed ARID1A deficient murine tumor models that exhibit anti-tumor immune phenotypes observed in ARID1A mutant human cancers, including increased CD8+ T cell infiltration and cytolytic activity. ARID1A deficient cancers upregulated an immunogenic interferon (IFN) gene expression signature, the ARID1A-IFN signature, associated with increased R-loops and cytosolic single stranded DNA (ssDNA). Overexpression of the R-Loop resolving enzyme, RNaseH2B, or the cytosolic DNase, TREX1, prevented ARID1A loss induced cytosolic ssDNA accumulation and upregulation of the ARID1A-IFN gene expression signature. Further, the ARID1A-IFN gene expression and anti-tumor immunity was driven by STING-dependent Type I IFN signaling, which was required for the improved responsiveness of ARID1A mutant tumors to ICB treatment. These findings define the molecular mechanism underlying anti-tumor immunity in ARID1A mutant cancers.

Metabolic Biomarkers for Circumventing Autophagy Inhibition in Cancer Cells

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Autophagy is a highly regulated and conserved metabolic process that is activated under cellular stress conditions. Cancer cells rely on autophagy to recycle damaged proteins/ organelle and sustain an elevated growth rate. In mouse models with established tumors, autophagy inhibition drastically reduces tumor growth and mortality rate prompting over 60 clinical studies with autophagy inhibitors like hydroxychloroquine (HCQ) in combination with a wide variety of targeted therapies. However, many of the clinical trials are starting to indicate that cancer cells can display intrinsic and acquired resistance to autophagy inhibition and patients who initially respond to autophagy inhibition treatments, later relapse. Moreover, data from these clinical studies fail to identify a biomarker that dictates autophagy-dependence. Recent studies from our lab suggests that rare autophagy-dependent cancer cells adapt to the complete loss of autophagy by altering their biology. In-depth analysis of these rare cancer cells can help us identify the metabolic pathways that compensate for loss of autophagy and will help us identify cancer cells that are sensitive to autophagy-inhibition.

One major obstacle to understand the mechanism of these adaptions is the lack of a tool that can regulate autophagy inhibition with high spatial and temporal specificity. Most genetic perturbation studies often take hours to days to be fully effective and by that time most of the signaling pathways have reached homeostasis. Such observation could be a result of the fact that cancer cells adapt too quickly against autophagy inhibition and these changes are impossible to detect with conventional genetic approaches. Therefore, to understand how quickly rare cancer cells adapt once autophagy is inhibited, we have developed an optogenetic system that uses blue light to inhibit the autophagy pathway. We demonstrate the Autophagic Snare for Abrupt Photo-inhibition (ASAP) tool can inhibit autophagy pathway within 5 minutes of blue light exposure in human cancer cells and the inhibitory effects increases with longer light exposure. As optogenetic inhibition of autophagy has not been previously tested, our system brings a novelty to the conventional genetic and pharmacological approaches of autophagy inhibition. In addition to developing the ASAP tool, we performed metabolic flux analysis with stable isotopic metabolic tracers to investigate which metabolic pathways are significantly modified after autophagy inhibition in autophagy dependent cancer cells. Next, we will utilize the novel ASAP tool to visualize the temporal kinetics of metabolic changes after rapid light-induced autophagy inhibition. Together, these experiments will help us identify metabolic biomarkers for autophagy dependent cancer cells and those prone to undergo adaptation to autophagy inhibition.

Targeting Mitochondrial Biogenesis and Antioxidant Pathways to Prevent T Cell Exhaustion

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T cells are major targets of cancer immunotherapy due to their critical role in killing cancer cells. However, due to chronic antigenic stimulation in the tumor microenvironment, T cells often acquire an exhausted phenotype that results in them being less responsive and effective. Given that the exhausted phenotype limits cancer immunotherapy responses, finding ways to alleviate this could have a huge impact on current cancer therapies. T cell activation is intrinsically linked to mitochondrial function, as T cells undergo various changes in mitochondrial metabolism, dynamics and biogenesis to support this transition. However, the exhausted phenotype is characterized by functional defects such as loss of self-renewal capacity, decreased production of cytotoxic effector molecules, upregulation of inhibitory immunoreceptors, and distinct metabolic, transcriptional and epigenetic alterations. Mitochondrial dysfunction and oxidative stress, caused by generation of mitochondrial reactive oxygen species (mtROS), are major drivers of T cell exhaustion. For example, T cells with lower mitochondrial membrane potential persist and maintain function longer due to lower mitochondrial ROS production and higher cellular antioxidant capacity. Furthermore, elimination of ROS with antioxidants partially restores T cell self-renewal and anti-tumor efficacy. Finally, exhausted T cells exhibit repression of PGC-1α, which acts as a transcriptional activator in promoting mitochondrial biogenesis and also regulates mitochondrial and cellular antioxidant capacity. Given that mitochondrial mass, membrane potential and ROS are inextricably linked, the overall goal of this project is to find avenues to combat T cell exhaustion based on manipulating specific mitochondrial biogenesis and antioxidant pathways in tumor-infiltrating T cells. We are utilizing MITO-Tag mice with endogenously tagged mitochondria to target mitochondrial biogenesis pathways. We have confirmed that this system can successfully detect increased mitochondrial content following T cell activation. These mice will be utilized to perform screens to identify genes and pathways required for 1) mitochondrial biogenesis in activated T cells and 2) maintenance of mitochondrial biogenesis and reduced mtROS in exhausted T cells. We are also determining if using a unique mouse model of mitochondrial mitohormesis to induce a heightened global antioxidant state will help to alleviate T cell exhaustion. Taken together these approaches will provide important new insights into targeting mitochondria and cellular antioxidant pathways to improve tumor immune responses and immunotherapy.

Exploring the Functional Landscape of Lipid Metabolism in Non-Small Cell Lung Cancer

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Lung cancer is the leading cause of cancer-related deaths worldwide. The most common form of lung cancer, non-small cell lung cancer (NSCLC) spans several major genetic subsets that exhibit distinct clinical behaviors and responses to therapies, including primary resistance to immune checkpoint blockade. Identifying genotype-driven phenotypic variations and understanding the underlying molecular mechanisms is of paramount interest, as it may uncover genotype-specific therapeutic vulnerabilities. Lipid metabolism plays a key role in tumorigenesis, as it supports membrane biogenesis, energy storage, and the generation of signaling molecules. While de novo synthesis of fatty acids is critical for lung tumor growth, lipid metabolism encompasses a complex array of interdependent processes, many of which remain poorly understood in NSCLC. Furthermore, the consequences of lipid metabolic reprogramming in NSCLC on the tumor microenvironment remain poorly appreciated. To enhance our understanding of the heterogeneity in lipid metabolic state across lung tumors and its potential to vary with genotype, I will perform lipidomic profiling across distinct genetic subsets NSCLC and elucidate the mechanisms that link genetic driver alterations to distinct lipid phenotypes. In parallel, I will leverage advanced *in vivo* functional genomics to map the functional landscape of lipid metabolism in NSCLC and define novel lipid metabolic dependencies. Finally, I will assess the impact of lipid synthesis by cancer cells on T cell-driven tumor clearance in a syngeneic NSCLC model. Collectively, these studies will establish a foundation for understanding lipid metabolism in NSCLC as it relates to genotype, reveal potential therapeutic targets among lipid metabolic processes, and identify potential lipid metabolism-centered strategies to bolster anti-tumor immunity.

Understanding the Molecular Mechanisms Underlying Acquired Resistance to Autophagy Inhibition in Pancreatic Cancer Cells

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Pancreatic ductal adenocarcinoma (PDAC) represents one of the deadliest forms of cancer. Therapies to treat PDAC are largely ineffective due to inherent and acquired chemoresistance. The cellular degradation pathway, autophagy, has been identified as a promising therapeutic target for pancreatic cancer patients. Although the autophagy inhibitor chloroquine can reduce cellular proliferation and tumor volume in PDAC cell lines and xenografted tumors, respectively, clinical trials combining autophagy inhibitors with current standard of care chemotherapeutics does not improve overall survival of patients with metastatic pancreatic cancer. This phenomenon is largely attributed to the development of cellular resistance to autophagy inhibition. Our lab recently demonstrated that genetic deletion of the autophagy gene ATG7 in autophagy-dependent breast and lung cancer cell lines results in initial cell death, followed by the expansion of surviving clones that exhibit normal growth rates and complete resistance to autophagy inhibition. However, it remains unclear whether resistance to autophagy inhibition is a result of general cellular adaptation in the face of impaired autophagy, or a selection of pre-existing inherently resistant clones. To address this gap in knowledge, we will utilize a technique known as "CellTag" that consists of the long-term labelling of cells with unique genomic barcodes allowing us to trace populations of cells derived from labeled parental lineages both *in vitro* and *in vivo*. By performing omics analysis at multiple timepoints during resistance acquisition and evaluating the enriched barcodes in the final resistant population, we will determine what cellular adaptations may facilitate acquired resistance and whether certain transcriptional and epigenetic profiles in the original untreated population provide an inherent advantage for autophagy inhibition resistance. Addressing these questions will aid in the development of effective therapy schemes for the treatment of autophagy-resistant cancers.

New Mouse Model Tools to Discover Effective Immune Therapies for Triple Negative Breast Cancer Metastases

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One of the reasons cancer is a lethal disease, is an insufficient understanding of how to cure patients with metastasis. This is especially true for Triple Negative Breast cancer (TNBC) where metastases are resistant to even the most promising immunotherapy treatments. Despite clinical studies that suggest new immune therapies that could cure metastatic disease, we lack experimental tools to establish mechanisms of action, safety, and optimization for curative anti-metastatic immune responses. The lack of experimental tools providing effective immune therapies slows progress in our ability to launch innovative clinical trials. Yet, mouse models offer an immune-competent setting to enable experimental testing for new safe, and effective treatments. However, such mouse models are still lacking, even more so when requiring the model has the same types of molecular presentation to the immune system as human cancers. Using the same cancer-causing events of BRCA1 and P53 loss as found in human tumors, we derived new mouse TNBC models that spontaneously metastasize to the lymph node, liver, and lungs. Bulk and single cell RNA-sequencing to obtain full transcriptomes and comparative bioinformatics, demonstrate immense parallels between these models and human tumors. Importantly, we also find parallel histology in these mouse models and human tumors. Comparing primary tumors and metastases, we identify unique tumor cell signaling in metastatic lesions that coincide with immune evasion. Like the metastases found in patients, these mouse metastatic TNBC lesions show commonalities in the immune microenvironments, including markers depicting similar deficits in T and B cell surveillance. Using these molecular data, we find key immune checkpoints that could enhance the immune systems recognition of the metastatic lesions. Thus, these new models and results will provide a powerful resource to immune-oncology and patients with opportunities to discover the requirements to successfully treat cancer with metastasis.

Evolution of Cells Progressing through Replicative Crisis to Immortality

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Transformation of a primary normal cell to a cancer cell requires multiple genetic and epigenetic alterations. One fundamental hallmark of human carcinogenesis is cellular immortalization by activation of a telomere maintenance mechanism (TMM) – either the activation of telomerase or the homologous recombination-driven "alternative lengthening of telomeres" mechanism. In normal cells the replication capacity is restricted by two proliferative barriers, replicative senescence, a permanent cell cycle block, and crisis, a state of excessive, innate immunity-driven, autophagy-dependent cell death. However, whereas senescence is well characterized, crisis, potentially the final barrier against human carcinogenesis, is poorly defined.
To analyze the crisis program in detail and gain insights in mechanisms favoring crisis escape, spontaneously escaping post-crisis clones of a large population of IMR90 E6E7 lung fibroblast crisis cells were isolated. Two populations of post-crisis clones emerged: Four of the nine post-crisis clones grew for over 300 days and showed telomerase activity, suggesting that these four clones are immortal. The remaining five post-crisis clones were initially proliferating, but then encountered a growth plateau marked by cell death, indicating that these mortal clones escaped crisis without TMM activation. Ongoing studies are aimed at the characterization of the genetic and transcriptional changes occurring in crisis and post-crisis cells. To this end we employed RNA-sequencing, whole-genome sequencing and to resolve the mutational heterogeneity we are currently performing single-cell DNA sequencing. Our analysis will help to comprehensively define the crisis barrier, the events that promote crisis escape and advance our understanding of the early stages in carcinogenesis.

PPARd Restricts Intratumoral Treg Function Through Negatively Regulating the CIITA-MHCII Axis

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Regulatory T cells (Treg) have been associated with poor clinical prognosis of multiple types of cancers due to their suppression of anti-tumor immunity. Although Treg depletion can lead to the rejuvenation of the patient's immune response to tumors, systemic Treg depletion induces autoimmune diseases such as type-1 diabetes and inflammatory bowel disease. Therefore, understanding how Treg cells adapt to the tumor microenvironment (TME) and how they suppress anti-tumor immunity is critical for the development of novel Treg-based cancer treatment. Peroxisome proliferator activated receptor δ (PPARd) is a member of the nuclear hormone receptor family, and plays a crucial regulatory role in fatty acids metabolism. Here, we uncover that ablation of PPARd in Treg leads to accelerated tumor growth with a more immuno-suppressive TME. Mechanistically, the loss of PPARd in Treg leads to upregulation of a set of genes involved in the antigen presentation pathway, including CIITA and MHCII. Deleting CIITA in PPARd deficient Tregs boosts anti-tumor immunity and inhibits tumor growth, indicating CIITA is a key regulator downstream of PPARd in Tregs. Moreover, overexpressing CIITA in Treg enhances its suppressive function, while knocking out MHCII reduces Treg suppressive function. Our study demonstrated that PPARd restrains tumor Treg function by downregulating the CIITA-MHCII pathway, and identified a previously unrecognized MHCII+ tumor Treg subset as a potent suppressor of anti-tumor immunity. Targeting this Treg subset could open a new avenue for boosting anti-tumor immunity while mitigating autoimmunity risk.

Deciphering the Role of AMPK in Pancreatic Cancer

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Pancreatic cancer remains a deadly disease, with a 5-year survival rate of 12%. A notable feature of pancreatic cancer is its extremely hypoxic environment. Tumor hypoxia induces adaptive changes in cancer cells and surrounded stromal cells, and is associated with cancer progression and therapy resistance. Efforts to better understand the pathways regulating metabolic adaptations in response to hypoxia will provide insight into pancreatic cancer progression and identify vulnerabilities that could be therapeutically targeted to improve patient survival. AMP-activating protein kinase (AMPK) is a master regulator of cellular and organismal metabolism that acts as a sensor of cellular energy by altering metabolism when energy levels are low. The Shaw lab and others have demonstrated that AMPK signaling provides cancer cells with the flexibility to adapt to metabolic stresses, such as hypoxia. Given that AMPK plays a key role in the adaptive response to hypoxia, we are interested in understanding the impact of AMPK on pancreatic cancer initiation and progression. Using orthotopic and genetically-engineered mouse models of pancreatic cancer, we have found that AMPK has tumor suppressive functions in pancreatic cancer. Here, we report the effects of deleting AMPK in a model of pancreatic ductal adenocarcinoma, in which KrasG12D and Trp53fl/fl are induced specifically in the pancreas leads to decreased overall survival. In parallel, we observed that orthotopically implanted AMPK-depleted pancreatic cancer cells developed larger tumors than their control counterparts. We plan to investigate the tumor suppressive function of AMPK in order to aid in our understanding of the metabolic stresses tumors face and how they may trigger adaptive changes in cancer cells.