CINCINNATI CANCER RESEARCH RETREAT 2019

HOSTED BY

Department of Cancer Biology
Division of Hematology/Oncology

Supported by

ORGANIZING COMMITTEE

Maria F. Czyzyk-Krzeska
Chenran Wang
Department of Cancer Biology

Atsuo Sasaki
Vladimir Bogdanov
Division of Hematology and Oncology
November 14th, 2019, Vontz Center, Rieveschl Auditorium

7:30-8:00  Continental Breakfast

8:00-8:30  WELCOME
Maria F. Czyzyk-Krzeska, Department of Cancer Biology
Jun-Lin Guan, Chair, Department of Cancer Biology
Pier Paolo Scaglioni, Chief, Division of Hematology & Oncology
Andrew T. Filak, Dean, UC College of Medicine

Session I  Chairs: Dave Plas and Krushna Patra

8:30-9:10  Jared Rutter, Department of Biochemistry, University of Utah School of Medicine
Mitochondria, Metabolism and Cellular Decisions

9:10-9:35  Pier Paolo Scaglioni, Department of Hematology & Oncology, University of Cincinnati
FASN Imposes a Targetable Metabolic Dependency on Mutant KRAS Lung Cancer

9:35-10:00 Maria Czyzyk-Krzeska, Department of Cancer Biology, University of Cincinnati
Landscape of Metabolic Subtype of Clear Cell Renal Cell Carcinoma From Tobacco Smokers

10:00-10:15 Coffee Break

10:15-10:55 Roberto Zoncu, Department of Biochemistry, Biophysics and Structural Biology, University of California, Berkeley
The Lysosome in Nutrient Sensing and Cellular Growth Control

10:55-11:20 Atsuo Sasaki, Department of Biochemistry, University of Cincinnati
Role of GTP-Energy Metabolism in Cell Functions and Cancers

11:20-11:45 Tom Cunningham, Department of Cancer Biology, University of Cincinnati
PRPS Enzymes: Linchpins of Nucleotide Synthesis and Vehicles for Dissecting Cancer Cell Metabolism

11:45-1:00 Lunch & poster viewing (posters 1-13)
### CINCINNATI CANCER RESEARCH RETREAT 2019

#### Session II: Chairs: Tom Cunningham and Atsuo Sasaki

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*Invited speakers: 40 min total (35 + 5)*  
*Local faculty: 25 min total (20 + 5)*  
*Short talks: 15 min total (10 + 5)*
Mitochondria, Metabolism and Cellular Decisions

Jared Rutter, PhD
HHMI Investigator, Professor of Biochemistry, University of Utah School of Medicine

Most differentiated cells convert glucose to pyruvate in the cytosol through glycolysis, followed by pyruvate oxidation in the mitochondria. These processes are linked by the Mitochondrial Pyruvate Carrier (MPC), which is required for efficient mitochondrial pyruvate uptake. In contrast, many proliferative cells, including cancer and stem cells, perform glycolysis robustly but limit fractional mitochondrial pyruvate oxidation. We sought to understand the role this transition from glycolysis to pyruvate oxidation plays in stem cell maintenance and differentiation. Loss of the MPC in Lgr5-EGFP positive stem cells, or treatment of intestinal organoids with an MPC inhibitor, increases proliferation and expands the stem cell compartment. Similarly, genetic deletion of the MPC in Drosophila intestinal stem cells also increases proliferation, whereas MPC overexpression suppresses stem cell proliferation. These data demonstrate that limiting mitochondrial pyruvate metabolism is necessary and sufficient to maintain the proliferation of intestinal stem cells. The impact of these effects on intestinal tumorigenesis will be discussed.
FASN Imposes a Targetable Metabolic Dependency on Mutant KRAS Lung Cancer

Pier Paolo Scaglioni, MD
Professor of Medicine, Division Director of Department of Hematology & Oncology, University of Cincinnati Cancer Institute

Mutant KRAS (KM) has been associated with metabolic reprogramming in KM lung cancer (KMLC). To determine the contribution of lipid metabolism to KMLC we performed functional studies with TVB-3664 (FASNi), a novel and selective inhibitor of fatty acid synthase (FASN), a CRISPR-CAS9 whole genome screening and mass spectrometry high-resolution lipidomic analysis of LC cells and microdissected KMLC specimens coupled with MALDI-imaging analysis (MSI).

Furthermore, we established that Acyl-CoA synthetase, ACSL3, is the main enzyme in KMLC responsible for the synthesis of fatty Acyl-CoAs, the substrates for beta-oxidation and for the synthesis of complex lipids. ACSL3 is required for the survival of KMLC both in vitro and in vivo.

We determined that KM upregulates FASN and the synthesis of palmitate and dramatically increases the cellular content of triglycerides, phosphatidylcholine and phosphatidylserine in KMLC. Accordingly, FASNi significantly inhibits cell proliferation of KMLC cells, inducing a G2/M cell cycle arrest and ferroptosis. Incubation with palmitate of KMLC cells completely rescues the deleterious effects of FASNi, ruling out possible FASNi off-target effects. On the contrary, LC cells harboring wild type KRAS are resistant to FASNi. We verified that FASNi treatment (oral gavage/60 mg/kg/daily), significantly impairs the tumor growth in KMLC mouse models. Metabolic flux analysis confirmed that FASNi effectively inhibits palmitate synthesis.

We conclude that KM orchestrates FA metabolism, dictating a dependency on FASN, which can be exploited in the treatment of KMLC. In particular, our preliminary data support the hypothesis that KMLC depends on de novo FA synthesis to feed the Land’s cycle to remodel peroxydated phospholipids to maintain phospholipid homeostasis and deflect ferroptosis. These results prompted us to design a phase II clinical trial of FASNi in KMLC patients. (NCT03808558).
Landscape of Metabolic Subtype of Clear Cell Renal Cell Carcinoma From Tobacco Smokers

Maria F. Czyzyk-Krzeska, MD, PhD
Professor, Department of Cancer Biology, University of Cincinnati College of Medicine

Tobacco Smoking (TS) is a dose-dependent risk factor in clear cell renal cell carcinoma (ccRCC), however the mechanisms of TS activities are not known. We investigated the landscape of ccRCCs and paired kidney tissues in a discovery cohort of never smokers (NS) and life-time current smokers (LTS) by transcriptomics, metallomics and metabolomics analyses. The study develops correlational analysis of metabolites’ bioinformatics approach to gain insights into the activity of metabolic pathways in human tissues, which are difficult to investigate by labeled isotope flux analysis. LTS ccRCCs show upregulation of genes encoding mitochondrial respiratory chain proteins and major reprogramming in essential metabolic pathways, including inhibition of glycolysis and activation of oxidative phosphorylation supported by the activity of malate aspartate shuttle. There are also major differences in metabolism of pyruvate, aspartate, glutamate, glutamine and histidine. Metallomics revealed dyshomeostasis of several metals, including increased levels of free inorganic As in tumors from LTS as compared to the organic, methylated As in NS. There is an imbalance in distribution of endogenous Zn and Cu reflected in the differential gene expression. This work identifies metabolic subtypes in ccRCC in LTS and NS with several different specific therapeutic vulnerabilities. This further supports the need for personalized medicine approaches in design of effective treatments.
The Lysosome in Nutrient Sensing and Cellular Growth Control

Roberto Zoncu, PhD
Assistant Professor, Biochemistry, Biophysics and Structural Biology, University of California, Berkeley

How do the nutrients we consume regulate our growth and homeostasis? Answering this question will help us understand not only how we develop, but also how we age and why we become susceptible to diseases as diverse as cancer, diabetes and neurodegeneration. Our research focuses on the lysosome, an organelle that is emerging as a key signaling node governing the balance between growth and catabolism. We are investigating how the lysosome relays nutrient-derived signals to the master growth regulator, mechanistic Target of Rapamycin Complex 1 (mTORC1) protein kinase. Using advanced live cell microscopy, in vitro biochemical assays, and high throughput protein and metabolite profiling, we are dissecting the rules that control mTORC1 activation at the lysosome and the triggering of its downstream anabolic and catabolic program. Our studies shed light on fundamental mechanisms of nutrient sensing, and point the way to novel strategies to correct faulty mTORC1 signaling in disease.
Role of GTP-Energy Metabolism in Cell Functions and Cancers

Atsuo T. Sasaki, PhD

Associate Professor, Department of Internal Medicine, Hematology & Oncology, University of Cincinnati College of Medicine

Guanosine triphosphate (GTP)—an evolutionarily conserved high energy metabolite — is utilized in all complex life to convey genetic information as well as to drive numerous enzymatic reactions in cells. Concentrations of GTP vary by tissue type, environment, and pathological condition and is regulated independently from the concentration of other energy molecules such as ATP. For example, many tumor cells have an elevated ratio of GTP against ATP. Recently, we demonstrated Type II phosphatidylinositol 5-phosphate 4-kinase β (PI5P4Kβ) as the first intracellular GTP sensor in mammalian cells. PI5P4Kβ converts GTP-concentration cues into the lipid second messenger signaling for metabolism and tumorigenesis (Sumita et al., Molecular Cell 2016). The finding revealed that changes in the GTP concentrations affect cell not only in a passive manner but also in an active manner via PI5P4Kβ, and suggest that some diseases such as cancer and diabetes could compromise the GTP energy-sensing system. More recently, we discovered a mechanism and functional significance of the GTP metabolic switch that promotes the enhanced anabolism and malignant growth of malignant brain tumors (Kofuji et al., Nat Cell Biol., 2019). In this retreat, we will introduce our approaches to further clarify the fundamental connection between GTP energy levels, cell functions, and human diseases, especially focusing on cancers.
The phosphoribosyl pyrophosphate synthetase (PRPS) enzyme plays an ancient and vital role in the metabolism of all cells by virtue of its production of phosphoribosyl pyrophosphate (PRPP), which is a necessary precursor for synthesis of purine, pyrimidine and pyridine nucleotides. Alterations in expression or mutation of either of the two ubiquitously expressed genes (PRPS1/2) encoding the PRPS enzyme underlie a host of human diseases. We have previously uncovered a mechanism critical for cancer development whereby MYC-driven lymphomas rely on enhanced expression of PRPS2 to drive supraphysiological production of nucleotides. Ongoing studies from our group are aimed at pinpointing the precise disruptions in cellular metabolism caused by PRPS2 loss that ‘short-circuit’ MYC-overexpressing cells. Results from these studies have revealed novel mechanistic connections between nucleotide production pathways and a variety of cellular processes; and identified potential rational combinatorial therapeutic approaches capable of thwarting cancers driven by the MYC oncogene.
mTORC2 and the Hexosamine Pathway in T Cell Development and Lymphoma

Estela Jacinto, PhD
Professor, Department of Biochemistry and Molecular Biology, Rutgers - Robert Wood Johnson Medical School

A highly proliferating cell reprograms its metabolism in order to meet the increasing demand for nutrients that are necessary for energy and macromolecule synthesis. A key signaling molecule that orchestrates metabolic reprogramming is mTOR. mTOR is part of two protein complexes, mTORC1 and mTORC2. While numerous studies have unraveled how mTORC1 is activated in highly proliferating cells in the presence of growth signals, how mTORC2 is activated and its role in metabolism is poorly understood. Recently, using cells in culture, we unexpectedly found that mTORC2 is robustly activated upon withdrawal of nutrients. The activation of mTORC2 modulates GFAT1, the key enzyme of the de novo hexosamine biosynthesis pathway (HBP), which produces metabolites necessary for protein and lipid glycosylation. By modulating GFAT1, mTORC2 maintains flux through the HBP to ensure survival during nutrient shortage. We used mouse models to determine how mTORC2 is activated in vivo and how its role in the regulation of GFAT1 is critical during the highly proliferative phases of specific normal T-cell subsets as well as in abnormal T-cell lymphoma. We will discuss how a disequilibrium in nutrient supply and demand in highly proliferating T cells escalates mTORC2 activation, thus remodeling the HBP. Our studies provide insights on how to more effectively starve and kill T-cell lymphoma and possibly other types of cancers that would be vulnerable to combined inhibition of mTOR and hexosaminebiosynthesis.
LSD1 Inhibition Sensitizes Sinonasal Squamous Cell Carcinoma Cells to Cisplatin

Layne Weatherford, PhD
Postdoctoral Fellow, Department of Hematology & Oncology, University of Cincinnati College of Medicine

Sinonasal squamous cell carcinoma (SNSCC) is relatively rare, accounting for less than 3% of all head and neck cancers. Despite intensive treatment, SNSCC is aggressive, with only approximately 50% of patients surviving beyond five years after diagnosis. Treatment typically involves a combination of surgery and radiation with or without cytotoxic chemotherapy, such as cisplatin. Little is known about genetic mutations that occur in SNSCC, and even less is known about potential driving mutations. Next-generation whole-exome sequencing on SNSCC tumor samples and adjacent normal tissue revealed that eight out of ten tumors contained mutations in the lysine methyltransferase gene KMT2C, which has specificity for H3K4 methylation, a mark associated with transcriptionally active promoters. We hypothesized that somatic mutations in a H3K4 methyltransferase may result in loss or reduction of function, which would decrease H3K4 methylation, giving SNSCC cancer cells a proliferative advantage via silencing of tumor suppressor and DNA damage response and repair genes. We aimed to indirectly target these mutations in SNSCC cells through inhibition of KMT2C’s druggable demethylase counterpart, LSD1, which has specificity for demethylation of H3K4me1/2. Given the high prevalence of KMT2C mutations observed in SNSCC tumors, we hypothesized that inhibition of LSD1 would prevent loss of H3K4 methylation and deactivation of key tumor suppressor genes in SNSCC and thus synergize with the cytotoxic drug cisplatin due to increased response to DNA damage.

LSD1 inhibitor treatment alone did not result in decreased cellular proliferation. However, LSD1 inhibitor in combination with cisplatin resulted in an enhanced decrease of proliferation for several cell lines compared to cisplatin or LSD1 inhibitor alone. We are currently in the process of genotyping the SNSCC cell lines for mutations in H3K4 methyltransferase genes. If it is confirmed that a high proportion of SNSCC tumors harbor mutations in H3K4 methyltransferases, further testing of LSD1 inhibitors or inhibitors of other H3K4 demethylases in in vivo models would be warranted with future possible translation to clinical trials.
Regulation of Immune Checkpoint Blockade Efficacy in Breast Cancer by FIP200: A Canonical-Autophagy-Independent Function

Syn Yeo, PhD
Department of Cancer Biology, University of Cincinnati College of Medicine

Immune checkpoint inhibitors (ICIs) have the potential to induce durable therapeutic responses but in breast cancer, response rates are modest and limited to particular subtypes. To expand the applicability of ICIs, we examined the role of an essential autophagy gene, FIP200, which has been shown to be important for tumor progression in mammary tumors. By employing genetic mouse models that specifically disrupt FIP200’s autophagy function or complete ablation of FIP200, we demonstrated that FIP200’s autophagy function was required for progression of PyMT driven mammary tumors. Interestingly however, FIP200’s non-autophagy function was responsible for increased T-cell recruitment and activation of the TBK1-IFN signaling axis. FIP200 was also found to interact with the TBK1 adaptor protein, AZI2, which was crucial for TBK1 activation upon FIP200 ablation. Accordingly, we showed that ablating FIP200’s non-autophagy function in combination with ICI therapy can lead to superior durable responses in immune-competent models of breast cancer. These insights could guide future development of therapeutic agents against FIP200 for combinatorial ICI therapies in non-responsive breast cancers.
Lipid Metabolism Reprogramming in Malignancy, from de novo Synthesis to Storage

Deliang Guo, PhD

Associate Professor, Department of Radiation Oncology, The Ohio State University James Comprehensive Cancer Center and Medical School

Lipid metabolism is greatly altered in human cancer. Our studies have shown that increased lipid uptake, storage and lipogenesis promote rapid tumor growth. Sterol regulatory element-binding proteins (SREBPs), a family of membrane-bound transcription factors in the endoplasmic reticulum, play a central role in the regulation of lipid metabolism. We demonstrated that SREBP-1 is highly upregulated by oncogenic signaling in human cancers to promote tumor growth, and revealed the underlying molecular mechanism that links glucose to lipid metabolism activation in cancer cells. We found that glucose induces N-glycosylation of SREBP-cleavage protein (SCAP), a key regulator of lipid metabolism, enabling SREBP-1 trafficking and nuclear translation to activate lipid synthesis and uptake for rapid tumor growth. Recently, we further identified that human brain tumors contain large amounts of special lipid-storing organelles, lipid droplets, which are rarely detected in normal brain tissues. Our data demonstrated that lipid droplets represent a unique signature in glioblastoma, and serve as a novel diagnostic and prognostic biomarker, which could also have significant implication for current brain tumor therapy. In summary, targeting altered lipid metabolic pathways has become a very promising anti-cancer strategy.
Oncogenic cAMP Signaling Network Reprograms Lipid Metabolism and Drives Pancreatic Cancer

Krushna Patra, PhD

Assistant Professor, Department of Cancer Biology

G-protein αs (GNAS) mediates receptor-stimulated cAMP signaling, a conserved pathway that integrates nutritional and hormonal cues with regulation of cellular metabolism and other processes. In many tissues GNAS-cAMP signaling is required to maintain anti-proliferative states via inactivation of key oncogenic mediators. Paradoxically, constitutively activating mutations in GNAS and other pathway components arise in multiple tumor types, although the mechanisms by which oncogenic GNAS incites tumorigenesis, its interplay with other cancer gene mutations, and its roles in the growth of fully formed cancers remain elusive. Here, we investigated the functions of activated GNAS in pancreatic tumorigenesis where concurrent GNAS and KRAS mutations define an important pancreatic ductal adenocarcinoma (PDA) subset. By developing genetically engineered mouse models (GEMMs), we show that inducible expression of mutant GNAS cooperates with oncogenic KRAS to drive pancreatic cancer and is critical for tumor maintenance at each disease stage. Moreover, by Proteomic, metabolomic, and functional studies demonstrated that GNAS drives tumor growth by driving a kinase cascade involving protein kinase A (PKA)-and Salt-inducible Kinase (SIK) critical for sensing hormonal and nutrient signals. This pathway reprograms cellular metabolism, potentiating lipid remodeling and fatty acid oxidation, supporting growth of GNAS mutant tumors but creating new targetable dependencies. Furthermore, comparison of KRAS mutant pancreatic cancers with and without GNAS mutations reveals striking differences in the circuitry and functional impact of this network, associated with dichotomous roles of the GNAS-PKA axis in growth control. Thus, our studies uncover GNAS-driven oncogenic mechanisms and demonstrate unanticipated metabolic heterogeneity among KRAS-mutant pancreatic neoplasms.
Autophagy Mediated Lipid Catabolism Facilitate Glioblastoma Progression to Overcome Bioenergetic Crisis

Chenran Wang, MD, PhD
Assistant Professor, Department of Cancer Biology

Activation of mTORC1 plays a significant role in glioblastoma development and progression. Although hyper-activation of mTORC1 is proposed to suppress autophagy, we recently revealed higher autophagy activity in Tsc-deficient tumor cells under energy stress. Glioblastoma cells could maintain high mTORC1 activity under energy stresses; however, whether and how autophagy is related to this capacity in glioblastoma is not investigated.

Kaplan-Meier analysis was performed to evaluate the prognostic value of mTORC1 upstream regulator and downstream targets and fatty acid oxidation (FAO) genes in glioblastoma patients. Metabolic and molecular assays were employed to explore the mechanisms to sustain mTORC1 activity through lipophagy in glioblastoma after 2-Deoxy-D-glucose (2DG) treatment. We generated an mTORC1 hyper-activated LN229 cell line by knocking down Tsc1. Growth curve assays and intracranial transplantation were used to analyze cell viability and tumor progression. The function of lipophagy to sustain mTORC1 activation was examined in animal models.

Kaplan-Meier analysis indicated a strong association of increased mRNA levels in mTORC1 targets and FAO pathway genes with poor prognosis of glioblastoma. We found that autophagy inhibitors or FAO inhibitors in combination with 2DG decreased mitochondrial OxPHOS, mTORC1 activity, ATP production, and survival of glioblastoma cells in vitro. The hyper-activation of mTORC1 in Tsc1-KD LN229 cells promoted tumor progression. The combination of 2DG with chloroquine (CQ) or 2DG with FAO inhibitors reduced tumor burden in mice.

The combination of glycolysis inhibitor with lipophagy downstream inhibitors effectively inhibited the progression of glioblastoma. These studies indicate selective lipophagy as an expedited clinical therapeutic target for future glioblastoma treatment.
The Dichotomy of AMPK – mTOR Metabolic Signaling in Mammalian Tissues

Biplab Dasgupta, PhD
Associate Professor, Pediatrics, Division of Oncology, Cincinnati Children's Hospital Medical Center

The catabolic energy sensor AMPK negatively regulates the anabolic nutrient sensor mTORC1, in part to protect mammalian cells during energy crisis. Downstream of AMPK, both TSC2 and RAPTOR regulate mTORC1 activity, while downstream of mTORC1, S6 kinase regulates a feedback loop that modulates mTORC2 and AKT activity. The extent to which AMPK, TSC2 and RAPTOR control mTORC1 and mTORC2 activity in tissues arising from different germinal layers is not well understood. Neither do we know how tightly the AMPK-mTORC1 axis is coupled in normal tissues basally and during physiological and pathological stress. For example, while the axis is firmly coupled in skeletal muscle in response to exercise stress, it is uncoupled in response to nutrient stress. In yet another example, while survival of nutrient-stressed fibroblasts is dependent to a large extent on the presence of a functional TSC2, it is largely independent of AMPK. Moreover, basal and stress AMPK-mTORC1 signaling in mesenchymal cells (fibroblasts) is distinct in many ways from that in neuroepithelial cells such as astrocytes. While examining AMPK and mTORC1 in the CNS, we observed both coexistence and spatial separation of AMPK and mTORC1 signaling in the normal mammalian brain and brain tumors. In the normal brain, neuronal mTORC1 activity overwhelms that in glia, while the amplitude of AMPK activity is reversed between these cell types. Yet, the AMPK-mTORC1 axis is coupled more tightly in neurons than in glia in vivo. In human brain tumors, we observed separation but also co-dependence on both AMPK and mTORC1 for optimal growth and survival. The dependence on AMPK was underscored by a deficiency in both glycolysis and mitochondrial OXPHOS when AMPK was inhibited not only in glioma cells but also in astrogial cells – one putative cell of origin of glioma. Our ongoing efforts are to understand the mechanistic nuances of AMPK-mTOR metabolic signaling in tissues during normal and stress physiology in vivo.
Signal Transduction Control of Metabolism and Survival in PTEN Deficient Glioblastoma

David Plas, PhD
Anna and Harold W. Huffman Endowed Chair in Glioblastoma Experimental Therapeutics, Department of Cancer Biology

Oncogenic driver events frequently activate apoptosis resistance mechanisms via the PI3K-Akt signaling pathway. The PI3K-Akt pathway activates glucose metabolism to support anabolic precursor metabolites and bioenergetic equivalents that are required for tumor growth and chemotherapy resistance. Aiming to restore physiologic apoptosis control and chemotherapy sensitivity in cancer cells, we have investigated the metabolic control mechanisms mediated by the ribosomal protein S6 kinases (S6Ks) in cancers of the brain, blood, and breast. Results have demonstrated a genetic requirement for S6K1 to sustain glucose metabolism in PTEN-deficient transformed cells. However, the activated tyrosine kinase BCR-ABL enabled cells to survive despite the loss of S6K1-driven glycolysis, through the rebound activation of fatty acid oxidation. Pharmacologic inactivation of using the S6K1-targeting multikinase inhibitor AD80 was selectively cytotoxic for PTEN-deficient glioblastoma and leukemia cells, suggesting that S6K1 is an oncodependent kinase in cells lacking PTEN. Based on kinome-wide analysis, a combination of specific inhibitors targeting S6K1 together with TYRO3-AXL-MERTK (TAM) family tyrosine kinases was found to selectively activate programmed cell death. Investigation of the metabolic effects of pharmacologic inhibitors indicated a decline in glucose metabolism together with interruptions in nucleotide biosynthesis. Thus the analysis of signal transduction control of metabolic function has revealed new approaches for counteracting PTEN-deficiency in glioblastoma.
Lysosomal Exocytosis Promotes Zn-dependent Reprogramming that Induces Cancer Initiating Properties in RCC Cells

Parul Aggarwal¹, Rita Verma¹, Megan E. Bischoff¹, Collin Wetzel¹, Julio Landero², Dina Secic², James Reigle¹,³,⁴, Katherine VandenHeuvel⁵, Nicolas Talbot¹, Jun-Lin Guan¹, David R. Plas¹, Jarek Meller³,⁴ and Maria F. Czyzyk-Krzeska¹

¹Departments of Cancer Biology; ²Metallomics Center & Rieveschl Laboratories for Mass Spectrometry, Department of Chemistry; ³Environmental Health, Division of Biomedical Informatics, University of Cincinnati; ⁴Department of Bioinformatics; ⁵Department of Pathology, Cincinnati Children's Hospital Medical Center; ⁶VA Research Service, Department of Veterans Affairs, Cincinnati, OH, USA

Microtubule Associated Protein 1 Light Chain 3 Gamma (MAP1LC3C, LC3C), a noncanonical human specific autophagic regulator, is induced by von Hippel-Lindau protein (VHL) and has tumor suppressing activity in clear cell renal cell carcinoma (ccRCC). In the process of identifying tumor suppressing functions of LC3C, we discovered that cells with LC3C knockdown demonstrate peripheral localization of lysosomes, secretion of active cathepsins into the media and presence of LAMP1, an integral lysosome membrane protein, on the cell plasma membrane surface. These are indicators of lysosomal fusion and exocytosis of lysosome content into the extracellular space. Using limiting dilution assay, we determined that a subpopulation of cells expressing surface LAMP1 has tumor initiating properties and forms fast growing tumors characterized by areas of necrosis. The tumor cells maintained LAMP1 protein on cell surface and were characterized by increased levels of NANOG1 and OCT4, transcription factors characteristic for stem-like cells. RNA-seq analysis revealed 1100 genes differentially expressed in cells with high vs. low LAMP1 expression on the plasma membrane, of which 40% genes were related to Zn function. Importantly, genes differentially expressed in cells with high and low LAMP1 show 40-50% similarity with genes differentially expressed in ccRCCs with high and low LAMP1 in TCGA-KIRC data base, based on the separation of ccRCCs into high and low LAMP1 expression using pan-cancer levels of LAMP1 as the threshold. Lysosomes can function in storage of Zn, and consistently with lysosomal exocytosis we found lower levels of Zn in cells with high LAMP1 expression. We also found lower levels of chromatin repression markers associated with Polycomb Repressor Complex 1 (PRC2), H3K27me3, SUZ12 and metal-inducible transcription factor 2 (MTF2) in the chromatin enriched fraction from cells with high LAMP1.

Overall these data indicate epigenetic reprogramming related to decreased levels of Zn caused by lysosomal exocytosis, leading to the induction of stem-like, tumors initiating phenotype. Metabolomic analysis indicates increased levels of succinate, and intermediates of glycolysis and pentose phosphate pathways, indicative features of higher proliferating tumor cells.

Supported by: NCI R01CA122346, R01GM128216, VA 2I01BX001110, DoD W81XWH-14-1-0347; AUA Research Scholarship (RT, PA) and UCCI Pilot Grant.
2.

Disrupting Nucleotide Economy in Hematologic Malignancies

Chanel Alford, Justyna Krupa and Tom Cunningham

Department of Cancer and Cell Biology

c-MYC (c-Myc) over-expression and dysregulation leads to increased proliferation and upregulated cellular metabolism, developments that cancer can exploit. c-Myc translocation is a genetic feature of many hematological malignancies including Burkitt’s lymphoma, Diffuse Large B Cell lymphoma, and Multiple Myeloma. Downstream of c-Myc activation are the pathways and processes that comprise nucleotide metabolism. In nucleotide metabolism the phosphoribosyl pyrophosphate synthetase enzymes 1 and 2 (PRPS 1 and 2) are the rate limiting step for the creation of pyridine, pyrimidine, and purine nucleotides. It has been shown that PRPS2 loss of function (LOF) in lymphomas with c-Myc overexpression partially but selectively induces apoptosis while PRPS1 LOF does not. This project aims to establish how nucleoside supplementation allow evasion of the induced synthetic lethality seen in Myc over-expressing lymphomas and determine whether PRPS2 LOF synergistically combines with nucleoside analogues to produce complete and selective cell death in c-Myc overexpressing cells. To answer these questions, we performed the Alamar Blue cell proliferation assay and Trypan Blue Cell Exclusion assay in the presence of nucleoside supplementation with DG75 and CA46 Burkitt’s lymphoma cell lines to determine growth kinetics. Additionally, we examined the expression of the enzymes responsible for salvage and recycling of existing nucleotides in both WT, PRPS1 LOF and PRPS2 LOF in DG75 and CA46 cell lines. In the experiments surrounding PRPS2 LOF and nucleoside analogues, the chemotherapeutics that were studied include 5-Fluorouracil, 6-Mercaptopurine, and 6-Thioguanine which are nucleobase analogues as well as Cladribine and Capecitabine which are nucleoside analogues. We also investigated the arabinose sugar analogues Ara-a, Ara-g, and Ara-c.
3. Scribble/Yap1 Polarization is Required for Hematopoietic Stem Cell Division and Fate

Mark J. Althoff, Ramesh C. Nayak, Shailaja Hegde, Ashley M. Wellendorf, Breanna Bohan, Marie-Dominique Filippi, Mei Xin, Q. Richard Lu, Hartmut Geiger, Yi Zheng, Maria T. Diaz-Meco, Jorge Moscat, Jose A. Cancelas

Division of Experimental Hematology and Cancer Biology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH; Hoxworth Blood Center, University of Cincinnati Academic Health Center, Cincinnati, OH Cancer & Cell Biology Program, University of Cincinnati College of Medicine, Cincinnati, OH Division of Immunobiology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH; Sanford-Burnham-Prebys Discovery Cancer Institute, La Jolla, CA

Yap1 and its paralogue Taz largely control epithelial tissue growth. We have identified that hematopoietic stem cell (HSC) fitness response to stress depends on Yap1/Taz. This effect depends on the predominant cytosolic co-polarization of Yap1 with Scribble. In HSC, Yap1 is scaffolded by Scribble C-terminus PDZ domains while Scribble N-terminus LRR domain co-polarizes with the Yap1/Taz negative regulator Lats1. Deletion of Scribble induces apoptosis of asymmetrically dividing HSC, expansion of self-renewing HSC, disruption of Yap1 polarization and inhibition of the polarization and activation of Cdc42, a major positive regulator of HSC quiescence. The combined loss of Scribble, Yap1 and Taz results in transcriptional upregulation of Rac-specific guanine nucleotide exchange factors, Rac activation and HSC fitness restoration. Scribble links Cdc42 and the cytosolic functions of the Hippo signaling cascade in HSC fate determination.
KRAS mutations (KM) are the most frequent genetic aberrations found in lung cancer (LC). However, no effective therapies for KMLC have been developed yet. Our group demonstrated that inhibition of Fatty acid synthase with TVB-3664 (FASNi) leads to promising results in KMLC preclinical models and early phase clinical trials in KMLC patients. Clinical experience demonstrates that chemotherapy and targeted therapies, including agents targeting metabolism, are often hampered by the emergence of acquired drug resistance. To gain insight into the mechanisms that underlie acquired FASNi-resistance and to establish the rationale for future combination therapy trials, we developed a preclinical model of acquired FASNi-resistance using KMLC cell lines. To this end, we treated KMLC cell lines with increasing concentrations of FASNi for 2 months. We confirmed by HPLC and 13C-labeled acetate flux analysis that FASNi was intracellularly retained and actively inhibited palmitate synthesis. As expected, FASNi-resistant cells were not affected by de novo lipogenesis ablation. FASNi-resistant cells display EMT and cancer stem cell (CSC) markers. Interestingly, the drug resistance phenotype was reverted when resistant KMLC cell lines were cultured in the absence of FASNi for 2 months. Such reversible reprogramming suggests that acquired FASNi-resistance is likely due to epigenetic changes rather than to genetic mutations. Our previous studies reported that expression of JumonjiC (JmjC) demethylases is often deregulated in multidrug-resistant lung cancer altering drug sensitivity. Thus, we tested whether JmjC proteins might mediate acquired resistance to FASNi. Indeed, we found that KDM6B is the only JmjC demethylase upregulated in FASNi-resistant KMLC cell lines with respect to parental cells. Accordingly, FASNi-resistant cells, but not the parental sensitive cells, showed a significant sensitivity to GSK-J4, a KDM6B selective inhibitor. Moreover, GSK-J4 treatment re-sensitized resistant cells to FASNi, leading to synergistic drug response. On the other hand, treatment with either inactive enantiomers or inhibitors of other JmjC family members did not elicit any significant change on cell viability, ruling out the possibility of off-target effects. Using a CRISPR-Cas9 screening we found that autophagy genes are essential for the survival of FASNi-resistant cells. Our hypothesis is that KDM6B removes the repressive markers H3K27me2/3 thus activating the transcription of genes involved in autophagy. Our findings suggest that the KDM6B demethylase accounts for FASNi-acquired resistance of KMLC and that inhibition of KDM6B might open a therapeutic opportunity for FASNi refractory patients.
5. FASN Imposes a Targetable Metabolic Dependency on Mutant KRAS Lung Cancer

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Mutant KRAS (KM) has been associated with metabolic reprogramming in KM lung cancer (KMLC). To determine the contribution of lipid metabolism to KMLC we performed functional studies with TVB-3664 (FASNi), a novel and selective inhibitor of fatty acid synthase (FASN), a CRISPR-CAS9 whole genome screening and a mass spectrometry high-resolution lipidomic analysis of LC cells and microdissected KMLC specimens coupled with MALDI-imaging analysis (MSI).

We determined that KM upregulates FASN and the synthesis of palmitate and dramatically increases the cellular content of triglycerides, phosphatidylcholine and phosphatidylserine. Accordingly, FASNi significantly inhibits cell proliferation of KMLC cells, inducing a G2/M cell cycle arrest and ferroptosis. Incubation with palmitate of KMLC cells completely rescues the deleterious effects of FASNi, ruling out possible FASNi off-target effects. On the contrary, LC cells harboring wild type KRAS are resistant to FASNi. We verified that FASNi treatment (oral gavage/60 mg/kg/daily), significantly impairs the tumor growth in KMLC mouse models.

Metabolic flux analysis confirmed that FASNi effectively inhibits palmitate synthesis.

We conclude that KM orchestrates FA metabolism, dictating a dependency on FASN, which can be exploited in the treatment of KMLC. In particular, our preliminary data support the hypothesis that KMLC depends on de novo FA synthesis to feed the Land’s cycle. Through the concerted actions of phospholipase A2 (PLA2) and lysophospholipid acyltransferases (LPCATs) KMLC remodels the pools of phospholipids synthesized through the Kennedy pathway to attain the proper fatty acid composition. These results prompted us to design a phase II clinical trial of FASNi in KMLC patients. (NCT03808558).
S6K1 Protein Correlates with Treatment Efficacy in Breast Cancer

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S6K1 is amplified in about 12% of TCGA breast cancers, is a signaling mediator downstream of mTOR Complex 1. Because it can function both upstream and downstream of the estrogen receptor α (ERα), S6K1 amplification can contribute to oncogenic estrogen signaling in breast cancer. We hypothesize that S6K1 amplification enables breast cancer cells to sustain proliferation during hormone therapy. Data show that genetic knock down of S6K1 reduces the progression of breast cancer into S phase. For breast cancer patients who have failed on Estrogen ablation therapy, an approved second-line treatment is the mTORC1 inhibitor, Everolimus, in combination with an ERα antagonist. We found that ERα cell lines revealed a wide range of S6K1 protein expression levels. We propose that patients with lower levels of S6K1, and therefore lower substrate phosphorylation, who complete a course of combination treatment will have better outcomes.
Selective and Non-canonical LC3C-dependent Tumor Suppressive Autophagy Targets for Lysosomal Degradation Postdivision Midbody Rings in Renal Cancer Cells

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Microtubule Associated Protein 1 Light Chain 3 Alpha, Beta, and Gamma (LC3A, B, and C) paralogs are required for the formation of a mature autophagosome. LC3C is an evolutionary late gene, present only in higher primates and humans. Its most distinct feature is a C-terminal 20 amino acid peptide cleaved in the process of glycine 126 lipidation. Postdivision Midbody Rings (PDMBs) implicated in cancer stem cell regulation are direct targets of LC3C autophagy. We report that, unlike canonical autophagy mediated by LC3B, LC3C autophagy requires noncanonical pre- and initiation complexes, including ULK3, UVRAG, RUBCN, PIK3C2A, and a member of ESCRT, TSG101. LC3C autophagy utilizes cargo receptor CALCOCO2 in a manner requiring the LIR motif on CALCOCO2 and the LIR-binding motif on LC3C. LC3C C-terminal peptide is necessary and sufficient to mediate LC3C-dependent selective degradation of PDMBs. The activity of the peptide requires intact proline 133 localized to a HIFα-like proline hydroxylation. VHL interacts with autophagic regulators. This work establishes a new noncanonical human specific autophagic program relevant to cancer stem cells.
Failure to Upregulate Calmodulin Underlies the Suppressed KCa3.1 Function and Enhanced Sensitivity to Adenosine in CD8+ T Cells of Head and Neck Cancer Patients

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The limited ability of the cytotoxic CD8+ T cells to infiltrate solid tumors presents a major roadblock to developing effective immunotherapy. Adenosine (Ado) accumulates in high concentrations in solid tumors where it contributes to the suppression of T cell function. We have previously shown that Ado reduces the chemotactic ability of peripheral blood CD8+ T cells (PBTs) from head and neck squamous cell carcinoma (HNSCC) patients by suppressing KCa3.1 channel function. Herein, we conducted experiments to elucidate the mechanism of KCa3.1 dysregulation in HNSCC PBTs. KCa3.1 channels are calcium-activated and require binding of calmodulin (CAM). PBTs were isolated from HNSCC patients and healthy donors (HD), and activated with CD3/CD28 antibodies. CAM levels decreased post-activation in HNSCC PBTs (by ~24%, n=7) while they increased in HD PBTs (by ~37%, n=6, p=0.001). To study whether CAM downregulation contributes to KCa3.1 dysfunction, we transfected HD PBTs with siRNA against CAM (siCam). siCam transfection decreased CAM expression and KCa3.1 currents, but not KCa3.1 expression. We then studied whether CAM downregulation affects the T cell chemotactic response to Ado in HD PBTs. Control (scrRNA-transfected) HD PBTs migrated towards CXCL12 in the presence of Ado, but downregulation of CAM abrogated their chemotactic ability in the presence of Ado (~71% inhibition, n=8, p<0.012). Activation of KCa3.1 channels with 1-EBIO restored the ability of siCam-transfected PBTs to migrate towards CXCL12 in the presence of Ado. Our data suggest that downregulation of CAM decreases KCa3.1 activity and suppresses chemotaxis in HNSCC PBTs which may contribute to their limited ability to effectively penetrate Ado-rich tumors.
9. Inhibiting the IL-6 and pSTAT3 Pathway in Natural Killer Cells for Treatment of Head and Neck Squamous Cell Carcinoma

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Head and Neck Squamous Cell Carcinoma (HNSCC) is the 6th most common cancer worldwide, and up to 50% of patients relapse despite intensive therapy. Immunotherapies, such as PD-1 inhibitors, which remove the blockade on cytotoxic immune cells, have drastically improved patient outcomes. However, only 20% of patients benefit from PD-1 blockade, the reason of which, is largely unknown. Natural Killer (NK) cells are an essential component of the immune system’s ability to attack cancer cells, and importantly, reduced levels of NK cells in HNSCC are linked to poor outcomes. Interleukin-6 (IL-6), a cytokine which activates the JAK2/STAT3 pathway, inhibits the cytotoxicity of NK cells, and increased levels of IL-6 are found in HNSCC patients with poor prognoses. pSTAT3 inhibition has been implicated in increasing NK cell activity, but the mechanism behind pSTAT3 and IL-6 activity with relation to NK cell function and HNSCC has not been extensively explored. We have demonstrated that a common anti-diabetic drug, metformin, with recently appreciated anti-cancer properties can downregulate pSTAT3 in HNSCC cells as well as increase the percentage of peripheral NK cells in HNSCC patients. Through flow cytometry, we identified that both CD56BrightCD16- and CD56+CD3+ cells are lower in HNSCC patient samples than healthy samples, but increased in number after metformin treatment. Utilizing Western Blot techniques, we have confirmed that metformin is a pSTAT3 inhibitor in HNSCC cells, but does not show significant pSTAT3 inhibition in PBMCs.

We have also shown that HNSCC cells treated with metformin have decreased IL-6 expression and that cells treated with IL-6 and metformin have lowered pSTAT3 as compared to IL-6 treated cells. Elucidating the pathway by which IL-6 and metformin interact and effect NK cells support for metformin, an already FDA approved drug, for use as a supportive drug for existing immunotherapies, such as PD-1 inhibitors.
Cancer cells are addicted to supra-physiological nucleotide levels to sustain unabated proliferation, a feature that renders them vulnerable to many chemotherapeutic agents. But the use of these agents lead to serious side effects and secondary ailments. Thus, there is a need to understand the mechanistic basis for such an addiction. Phosphoribosyl pyrophosphate synthetase isoforms (PRPS1 and PRPS2) are key rate limiting enzymes catalyzing the conversion of ribose-5-phosphate to phosphoribosyl pyrophosphate (PRPP). In normal cells, optimum rate of nucleotide production is maintained by regulating PRPS1 enzyme activity via end-product feedback inhibition and low expression of feedback-refractory PRPS2. Mutations in PRPS1 imbue developmental disorders and drive resistance to chemotherapy in relapsed B-ALL, while c-Myc mediated PRPS2 upregulation drives enhanced nucleotide production in Myc-transformed cells. Therefore, regulating PRPS activity and expression is critical for normal physiology. Previous studies on PRPS activity report PRPS1/2 enzymes exist as homodimers arranged in a hexameric conformation but our preliminary data suggests the possibility of a heterodimer arranged in a multimeric complex with other associated proteins, thereby, opening up the idea of stoichiometry and composition of PRPS complex in regulating its enzymatic activity. Likewise, PRPS2 expression is well-known to be regulated by a unique PRTE (pyrimidine-rich translational element), located within the PRPS2 5’UTR (untranslated region). We have identified a putative PRPS1 5’uORF (upstream open reading frame) within its 5’UTR (untranslated region) potentially regulating PRPS1 translation. This study, thereby, aims to explore whether PRPS1 and PRPS2 5’UTR elements co-evolved to acquire different structural and biochemical properties. Thus, an understanding of the dichotomy in the activity and expression of the two isozymes will not only help us in understanding how stoichiometric changes influence nucleotide production but also lead us towards a safer and precision based anti-cancer therapy.
11. Novel Combination Therapy Leads to Tumor Regression in Rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in pediatrics, accounting for about 7% of all pediatric tumors. RMS is highly metastatic, and patients with metastatic RMS have less than a 20% survival rate. Recurrence, metastasis, and therapeutic resistance are the main causes of treatment failure in patients with metastatic disease, indicating more effective treatment strategies are urgently needed. FOXM1 is a transcription factor overexpressed in a variety of human cancers, including RMS, and leads to proliferation of cancer cells. Robert Costa Memorial Drug-1 (RCM-1), is a small-molecule inhibitor of FOXM1, recently identified by high-throughput screening. Vincristine (VCR) is a chemotherapy commonly used for RMS patients as a single agent, or in combination at all RMS stages in the clinic. Both RCM-1 and VCR target cells actively in the cell cycle as single agents, and we show that the combination of both RCM-1 and VCR lead to a robust decrease of proliferation, and increased apoptosis of cancer cells both in vitro and in vivo.
S6K1 and TAM RTKs as Therapeutic Targets in PTEN Deficient Glioblastoma

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Glioblastoma multiforme (GBM) is a highly aggressive stage IV tumor; the loss of tumor suppressor gene PTEN is considered a major driving mutation in over 30% of glioblastoma cases. We have previously shown that the genetic loss of ribosomal protein S6 Kinase 1 (S6K1) reduces apoptosis resistance in PTEN-deficient GBMs. A kinome-wide analysis to identify targets that would cooperate with S6K1 to induce further cytotoxicity revealed a potential synergistic interaction between S6K1 and TAM receptor tyrosine kinases (RTKs) targeting.

Overexpression of TAM family RTKs is frequently observed in multiple cancers, including glioblastomas, and has been linked to both poor prognosis and chemoresistance. We investigated the activity of pharmacologic S6K1 inhibition alone and in combination with TAM inhibitors for effects in PTEN-deficient GBM cells. The S6K1 selective inhibitor LY2584702 was ineffective as a cytotoxic agent; however, when combined with TAM kinase inhibitor BMS777607, a selective cytotoxic response was induced in PTEN-deficient cells and sphere cultures. PTEN-deficient patient derived xenograft (PDX) neurospheres were sensitive to dual S6K1 and TAM targeting. This PDX model was further validated in vivo where combination therapy extended survival time of mice implanted with intracranial PDX tumors. These results reveal that combination targeting of S6K1 and TAMs is a viable therapeutic for the treatment of PTEN-deficient glioblastoma.
Pembrolizumab Alters $K^+$ Channel Function to Modulate $Ca^{2+}$ Fluxes in T Cells of Head and Neck Cancer Patients

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Pembrolizumab has been approved as the first-line treatment of patients with metastatic head and neck cancer (HNC). Pembrolizumab blocks the interaction between immune checkpoint, PD-1 (programmed death receptor-1) and its ligand (PD-L1/PD-L2). This interaction reduces T cell receptor (TCR)-stimulated $Ca^{2+}$ fluxes. Ion channels regulate $Ca^{2+}$ entry in T cells downstream to the TCR (store-operated $Ca^{2+}$ entry, SOCE). We hypothesized that Pembrolizumab modulates $K^+$ channel activity and increases SOCE. We studied the effect of Pembrolizumab and PD-L1-Fc on SOCE in activated CD8+ peripheral blood T cells (PBTs) of HNC patients and healthy donors (HD) using Thapsigargin (TG). Additionally, we tested the effect of Pembrolizumab on KCa3.1, Kv1.3 and $Ca^{2+}$ release activated $Ca^{2+}$ (CRAC) channels in HNC PBTs using patch-clamp electrophysiology. TCR-mediated $Ca^{2+}$ fluxes were assessed in PD-1-expressing NFAT-reporter Jurkat cells. In these cells, Pembrolizumab increased the number of cells that responded to TCR stimulation with a sustained increase in $Ca^{2+}$ (30%, $p<0.001$). HNC PBTs showed lower SOCE compared to HDs ($p<0.001$). Pembrolizumab increased the SOCE in PBTs of HNCs and HDs (+/- PDL-1-Fc, $p<0.02$, $p=0.001$). PD-L1-Fc decreased KCa3.1 conductance in HD PBTs ($p<0.001$). Pembrolizumab significantly increased KCa3.1 and Kv1.3 channel conductances but not the activity of CRAC channels in PBTs of HNCs. Expression of ion channels (Orai1, STIM1, Kv1.3, KCa3.1 and TRPM7), T-cell exhaustion markers (PD-1, LAG-3 and TIM-3) and T-cell activation marker (CD69) in PBTs of HNCs were unchanged by Pembro. These data suggest an effect of Pembrolizumab on ion channels may contribute to the improvement of T cell function. (DoD CA160714; 2-R01-CA95286)
FOXF1 Promotes Tumor Vessel Normalization and Prevents Lung Cancer Progression

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Lung cancer is the leading cause of cancer-related mortality worldwide. The 5-year survival rate is less than 20\%, emphasizing a need to improve existing therapies. Solid tumors, including lung tumors are characterized by abnormal vasculature that plays a crucial role in tumor progression. Identification of factors that regulate the abnormal tumor vasculature will improve our understanding of tumor vascular biology and aid in designing novel therapeutic targets to normalize the tumor vasculature. Forkhead box F1 (Foxf1) is a transcription factor expressed by normal lung endothelial cells (ECs), and a known key regulator of embryonic development.

Through whole transcriptomic analysis and immunohistochemical analysis, we identified Foxf1 was downregulated in TECs in lung cancer patient samples and mouse models of lung cancer. Furthermore, lower Foxf1 expression correlated with poor overall survival of lung cancer patients. Through orthotopic injection of lung cancer cells in an inducible EC-specific Foxf1 heterozygous mouse model (endFoxf1+/−) and inducible EC-specific Foxf1 overexpression (endFoxf1OE) mouse model, we observed that Foxf1 in ECs inhibited lung tumor growth and metastasis. Using gene expression and immunohistochemical analysis, we observed that Foxf1 in ECs promotes vascular normalization and in turn survival of anti-tumor immune cells. To determine the mechanism by which Foxf1 regulates vascular normalization, we performed RNA-seq, and observed down-regulation of Wnt/\(\beta\)-catenin signaling pathway in Foxf1-deficient TECs. Using in vitro assays, we found that Foxf1 regulates Wnt/\(\beta\)-catenin signaling pathway in ECs through its upstream receptor Frizzled 1. Thus, FOXF1 regulates vascular normalization and lung cancer progression through Frizzled 1/ Wnt/\(\beta\)-catenin signaling pathway in ECs.
Targeting Metabolic Vulnerabilities Driven by Ron Receptor Expression in Progressive and Recurrent Breast Cancer

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Despite advances in clinical detection, molecular subtyping, and targeted therapeutics, breast cancer (BC) is the second leading cause of cancer-related death in women. Recurrent and metastatic disease is the underlying cause of BC mortality. Due to fear of recurrence, BC is the most overtreated cancer creating significant ethical and financial burdens. Through a discovery-based genomics approach, the RON receptor tyrosine kinase was identified as a predictor of BC recurrence. We and others have shown that RON overexpression occurs in more than half of all BCs and that RON levels are a predictor of metastasis and poor survival. Through evaluation of human breast tumor tissue, we provide the first line of evidence that RON overexpression occurs independent of molecular subtype and provide prognostic evidence of RON expression as a biomarker of BC recurrence. Further, we identified glycolysis and cholesterol biosynthesis, two potentially therapeutically targetable processes, as being significantly upregulated by RON. To test the efficacy of reducing cholesterol as a therapy for BC with RON expression, we treated two independent, spontaneous murine breast cancer models with inhibitors directed at RON and/or cholesterol biosynthesis (Atorvastatin). Our studies show that either RON or cholesterol inhibition reduce BC progression and that combination treatments blocked progression further. Current studies are underway to determine the mechanisms by which RON promotes metabolic pathway dependency and to directly test in a novel murine model of BC recurrence if targeting these pathways provides a survival benefit. We anticipate that our studies will serve as the scientific underpinnings for rationally designed new combinatorial therapeutic strategies to decrease the morbidity and mortality associated with advanced breast cancer.
Increased TGF-β Signaling Drives Bone Marrow Failure After Acute Inflammatory Stress

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Bone marrow failure (BMF) syndromes, such as myeloid dysplastic syndrome (MDS), can arise from acute or chronic inflammatory events that cause hematopoietic stem and progenitor cells (HSPCs) to favor differentiation over self-renewal, driving exhaustion of the HSPC pool and cytopenia in different blood cell lineages. Our lab has shown increased levels of active TGFβ1 (aTGFβ1) in transplanted murine HSPCs compared to non-transplanted HSPCS, which drove differentiation over self-renewal in HSPCs. Preliminary data using a conditional aTGFβ1-overexpressing transgenic mouse model (Tg-Cre+) surprisingly showed chronic MDS-like phenotypes starting 3 months and up to 9 months after acute polyinosinic:polycytidylic acid (pIC)-driven inflammation. Analysis of Tg-Cre+ HSPCs 3 months after pIC stress suggests that a MAVS-NLRP3 inflammasome signaling axis downstream of aTGFβ1-overexpression drives the BMF phenotypes in the mouse model, indicated by increased MAVS and PYCARD activation, as PYCARD is an essential subunit of the NLRP3 inflammasome. We therefore hypothesize that in response to acute inflammatory stress, HSPCs activate a non-canonical MAVS-NLRP3 signaling axis that drives differentiation at the expense of self-renewal. Preliminary data supporting this hypothesis using MAVS +/- mice stressed with pIC show that MAVS haplo-insufficiency causes reduced activation of PYCARD and the NLRP3 effector caspase-1, 48 hours after stress.
Exploiting Metabolic Dependencies in Diffuse Intrinsic Pontine Glioma

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Diffuse intrinsic pontine glioma (DIPG) is an incurable brainstem malignancy in children with median survival less than 1 year and 5-year overall survival only 2 percent. Little progress has been made in treating this deadly disease due to its inoperable location and treatments aimed at targets defined in adult gliomas. Despite recent advances in genetic characterization of DIPGs there are still no targeted therapies that significantly improve overall survival. Additionally, there is no literature describing the metabolic alterations for these tumors, and as documented in other cancers, metabolic reprogramming controls every aspect of tumor biology. We recently generated a metabolic profile for DIPG elucidating an upregulation in purine metabolism.

Normally nucleotide salvage maintains cellular purine levels by recycling degraded bases, however the de novo pathway is activated when purine levels are depleted. Furthermore, the transient clustering of de novo enzymes into complexes known as purinosomes increases flux through this pathway. Importantly, the last two steps in de novo purine synthesis are catalyzed by the enzyme AICAR-transformylase/IMP cyclohydrolase (ATIC), which is overexpressed in our patient derived cell lines and in tumor tissue from patients with DIPG. Although mutations in the ATIC gene negatively impacts purinosome formation, the effect of ATIC overexpression is not known. Elucidating purinosome assembly and de novo purine synthesis as a function of ATIC expression will provide insight into purine metabolism in cancer and more specifically in DIPG tumor biology. The development of pharmacological approaches to target this gene will lead to novel treatment strategies for children plagued by this disease. Our preliminary data indicates DIPG cell lines derived from patient tumors are sensitive to ATIC inhibition and the effect of ATIC overexpression on purinosome assembly is currently being investigated. In vivo work is in progress to validate our metabolic profile and to determine the efficacy of targeting ATIC in orthotopic xenograft and genetically engineered mouse models for this disease.
18. **Pembrolizumab Treatment Increases K⁺ Channel Function and Calcium Fluxes in Cytotoxic T Cells of Head and Neck Cancer Patients**

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Head and neck cancer (HNC) is the sixth most common cancer worldwide with a five-year survival of 50%. Immunotherapy is emerging as a promising treatment modality in HNC. Pembrolizumab, an anti-PD-1 antibody, is an immunotherapy currently approved in metastatic HNC and in clinical trials for curative intent. Although there are promising clinical responses to Pembrolizumab, its effect on cytotoxic CD8+ T cell function is not understood. T cell cytotoxicity is critically important in the elimination of tumors and in the immunosuppressive tumor microenvironment cytotoxicity depends on ion channel activity which is defective in HNC patients. Therefore, to elucidate possible mechanisms of Pembrolizumab, we studied potassium channel (KCa3.1 and Kv1.3) functionality and calcium fluxes in CD8+ peripheral blood T cells (PBTs) of naive HNC patients before/after Pembrolizumab treatment (n=22) as well as functionality of tumor infiltrating lymphocytes (TILs). Pembrolizumab-treated patients were categorized as responders or non-responders based on pathological response. Untreated HNC patients were used as controls (n=13). We then performed electrophysiological experiments, calcium flux assays, and flow cytometry. We observed that Pembrolizumab increased KCa3.1 currents in PBTs as compared to untreated PBTs (30.1%, p=0.017). Additionally, Kv1.3 currents and calcium fluxes increased in Pembrolizumab-treated TILs as compared to untreated, respectively (64%, p=0.002; 36.8%, p=0.006). Moreover, after treatment, Kv1.3 currents and calcium fluxes increased in responder’s PBTs, respectively (22.1%, p=0.047; 13%, p<0.001). These data support a role of ion channels and calcium fluxes in patient response to Pembrolizumab and could lead to better understanding of the Pembrolizumab mechanism of action/resistance. (DoD CA160714; 2R01 CA95286; T32CA117846)
Essential Role of FAK in the Growth and Progression of MMTV-Wnt1 Driven Basal-like Mammary Tumors

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Breast cancer is a heterogeneous disease. Hence, stratification of patients based on the subtype of breast cancer is a key to its successful treatment. Among all the breast cancer subtypes, Basal-like breast cancer is the most aggressive subtype with limited treatment options.

Interestingly we found Focal Adhesion Kinase (FAK), a cytoplasmic tyrosine kinase is highly over expressed and activated in basal-like breast cancer. To understand the role of FAK in this subtype, we generated mice with conditional deletion of FAK and a knock-in mutation in its kinase domain in WNT1 driven basal-like mammary tumors. We found that in the absence of FAK or its kinase function, growth of the tumors is significantly suppressed. Furthermore, immunohistochemical analyses of Cleaved Caspase 3 revealed that loss of FAK results in induction of apoptosis. To further investigate the mechanism by which FAK regulates survival of the WNT1 driven tumor cells, we compared the transcriptomic profile of the tumor cells with and without FAK. Gene-set enrichment analysis from the transcriptomic data suggested mTORC pathway to be downregulated upon loss of FAK. Immunoblot analyses further confirmed that absence of FAK results in reduction of AKT and downstream mTORC pathways. We also found that inhibition of AKT and mTORC pathways induces apoptosis, indicating the importance of these pathways in regulating cell survival. In addition, we found that in the absence of FAK, the tumor cells were sensitive to ER stress inducing agents unravelling a novel combination therapy for the treatment of basal-like tumors. In summary our studies show that in a basal-like tumor model, FAK is required for survival of the tumor cells and can serve as a potential therapeutic target.
S6K1 and S6K2 Networking with the AXL Tyrosine Kinase in PTEN-deficient Glioblastoma

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Glioblastoma (GBM), the most lethal type of malignant brain cancer in adults, sustains frequent mutations and/or deletions in the tumor suppressor gene PTEN, resulting in the sustained activation of the S6 kinases (S6Ks). We found that combining the LY-2584702 inhibitor of S6K1 with the BMS-777607 inhibitor of the AXL receptor tyrosine kinase (RTK) was selectively cytotoxic for PTEN-deficient GBM. Tumor cell cytotoxicity in response to combination therapy corresponded with decreased pyruvate entry to the TCA cycle through pyruvate carboxylase, and decreased nucleotide biosynthesis. Treating S6K1-/- vs. S6K2-/- GBM cells with single agent AXL inhibitor indicated that S6K2 is a major mediator of BMS-resistant signaling. Possible feedback mechanism showing crosstalk between S6K2 and Axl was mediated by Gas6 stimulation. In short, our data support unique features of S6K1 and S6K2 regulation by PTEN and AXL receptor tyrosine kinase, suggesting the importance of both kinases as clinical targets in GBM.
21. Hyperglycemia, Protein Glycosylation and Cancer – Is There A Connection?

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Cancer risk imposed by external factors can be as high as 70%. Epidemiological studies underscore Type 2 diabetes (T2D) and associated insulinemia and hyperglycemia as key risk factors for a variety of human cancers. T2D is characterized by high levels of two pro-growth hormones insulin and insulin-like growth factor (IGF1). Very little is known if tissue glucose utilization per se, independent of insulin, affects proliferative fidelity of mammary epithelial cells. Dissecting the role of glucose per se (beyond its traditional role as a fuel for glycolysis) in cancer development and progression is challenging. O-GlcNAcylation is a glucose-dependent, dynamic and reversible process that targets cytoplasmic and nuclear proteins. It is catalyzed by a single enzyme O-GlcNAc transferase (OGT) that transfers N-acetylglucosamine from UDP-GlcNAc to protein substrates. O-GlcNAc is removed by N-acetyl-beta-glucosaminidase (OGA). Because glucose directly feeds the hexosamine pathway and protein O-GlcNAcylation is elevated in cancer, we hypothesize that aberrant O-GlcNAcylation and misregulation of cell cycle and DDR proteins in hyperglycemia may cooperate with cancer predisposing mutations or environmental carcinogens to accelerate initiation and progression of cancer. Our preliminary studies in mammary epithelial cells showed that O-GlcNAc levels increase with increasing glucose concentrations, and it is levels change rapidly within minutes when glucose levels are altered. Our aims are to determine if high glucose utilization enhances OGT-dependent proliferation of normal epithelial cells; if it cooperates with cancer predisposing mutations; and if it alters O-GlcNAcylation and function of proteins involved in cell cycle and DNA damage response (DDR). We have also established an oral sucrose water-induced hyperglycemia model in C57BL6 mice. We will determine the consequences of hyperglycemia on systemic and epithelial tissue-specific O-GlcNAcylation, hyperplasia, DDR and cell division. We also plan to replicate our hyperglycemia model in several mouse models of cancer.
Epithelial Ron Promotes M2 Macrophage Activation to Drive Prostate Tumor Growth and Progression

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The incidence of advanced prostate cancer (PCa) in American men has grown by 72% within the last decade yet current treatments fail to effectively treat advanced disease. Our studies focus on the Ron receptor tyrosine kinase as a novel target for PCa treatment. Ron is overexpressed in PCa, and Ron expression increases with disease severity in both human PCa and in mouse PCa models, suggesting that Ron drives progression to advanced PCa. To test the significance of Ron in PCa, we previously showed that ubiquitous loss of Ron signaling in the well-established Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) PCa mouse model significantly reduced prostate tumor growth and was associated with modulations in the prostate tumor microenvironment. We next sought to interrogate the contribution of prostate epithelial-specific Ron signaling to PCa. We used Cre-Lox technology to generate TRAMP mice containing a conditional loss of Ron selectively in prostate epithelial cells, and we harvested prostates and distal organs from mice at 30 weeks of age. Our results show that epithelial Ron loss is critical for tumor growth in TRAMP mice. In our analyses, we also identified a significant influx of macrophage to the prostates of mice with epithelial Ron loss, increased intratumoral iNOS staining, and a robust increase in markers of alternative (M2) macrophage activation. Based on these results, our overarching hypothesis is that epithelial Ron expression promotes PCa growth and progression in part through the recruitment and alternative activation of macrophages to the prostate tumor microenvironment. We performed in vitro co-culture experiments to assess the ability of epithelial Ron to drive macrophage activation. Our preliminary data suggest that loss of epithelial Ron suppresses antitumor M1 macrophage activation markers and promotes the expression of tumor-supporting M2 macrophage activation markers. These studies suggest the that the Ron receptor coordinates immunosuppressive mechanisms in prostate tumor cells which serve to dampen antitumor immunity and promote PCa. These studies implicate Ron targeting as a powerful therapeutic tool with broad utility for understanding and refining therapeutic strategies to treat advanced PCa.
Chronic liver injury and inflammation are often associated with hepatocellular carcinoma (HCC) pathogenesis, as they often promote the activation of the DNA damage response (DDR) pathways. Therefore, genomic alterations and protein modifications of DDR genes regulate their activity and fate and can contribute to disease susceptibility. Our group has shown that Bir repeat-containing ubiquitin conjugating enzyme (BRUCE), an E2 sub conjugase, is necessary for localization and activity of two major DDR kinases. Additionally, BRUCE heterozygous mice develop spontaneous HCC after 10 months of age and over 50% of human chronic liver disease patient samples have decreased BRUCE expression. This data suggests that BRUCE may play a protective role against liver disease progression. Yet the influence of BRUCE in HCC pathogenesis remains unclear. To gain insight on the influence of BRUCE loss on HCC pathogenesis, we have generated a BRUCE liver-specific knockout (LKO) mouse model. Our mice WT and LKO were subjected to drug-induced liver injury with the DNA damaging environmental carcinogen diethylnitrosamine (DEN). LKO mice develop tumors at a 100% incidence as compared to the 80% incidence observed in WT mice. LKO mice developed liver tumors at an earlier rate as compared to their WT counterparts. Interestingly, BRUCE Alb-KO mice also have increased cell proliferation, as well as nuclear β-catenin localization in tumor and non-tumor tissue. We suspect that the loss of BRUCE in HCC patients is associated with a more exacerbated HCC phenotype and can be correlated with the aberrant activation of β-catenin, a common driver of HCC in humans. Dissecting the importance of BRUCE in the liver, as well as its relationship to a common HCC driver gene, β-catenin, will provide a therapeutic window for the subset of HCC patients with reduced BRUCE expression and aberrant β-catenin activation.
Inducible Correction of a RUNX1 Mutation of Human AML Causes a Switch of AML to B-ALL

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Background: Point mutations of RUNX1 gene are frequently seen in myeloid malignancies such as myelodysplasia (MDS) and acute myeloid leukemia (AML), and are associated with unfavorable clinical outcomes. AML patients bearing RUNX1 mutations have a significantly lower complete remission (CR) rate compared with patients with wild-type RUNX1 (30\% vs. 73\%), whereas in contrast, the CR rate of ALL patients, in which RUNX1 is rarely mutated, are at 92\%. It remains unclear whether RUNX1 mutations found in AML patients are causal to AML maintenance, and if RUNX1 mutations serve as a valid therapeutic target in the treatment of such therapy-resistant AML.

Experimental Approach: The RUNX1 mutations are found frequently associated with MLL-partial tandem duplication (MLL-PTD) in AML. We have developed two complementary mouse models that express a MLL-PTD knock-in mutation together with a RUNX1 S291fsX300 mutation found in AML patients: in the first model RUNX1 S291fsX300 was introduced into the bone marrow cells of the MLL-PTD knock-in mice by retrovirus transduction, while in the second model the tetracycline-inducible RUNX1 S291fsX300 mutation was knock-in at the Collagen a1 locus and the mice was crossed to MLL-PTD knock-in. In the second model, the mutant RUNX1 protein is only expressed when the mice were fed with doxycycline containing food and the RUNX1 mutant is absent or ‘corrected’ upon doxycycline withdrawal. BM cells of these mice, as well as WT and MLL-PTD controls, were transplanted to syngeneic mice and the recipients were tracked for disease development and progression at bi-monthly intervals. The doxycycline induction or withdrawal was carried out after secondary transplant, and the expression of RUNX1 S291fsX300 was monitored by a built-in GFP reporter and verified by RT-PCR. Under tetracycline induction, the RUNX1 S291fsX300 and MLL-PTD double mutation bearing mice developed a spontaneously AML and had a survival time 6-10 months after transplantation, and they showed symptoms of MDS/AML or AML, including thrombocytopenia, anemia, leukocytosis, splenomegaly, abnormal BM and spleen cell morphologies. We transplanted the AML mouse bone marrow to secondary recipients, and observed them with or without continuing doxycycline induction. By using the O-propargyl-puromycin to track the newly synthesized proteins, we found that the RUNX1 mut-on and RUNX1 mut-off mice showed comparable protein synthesis rate at full-blown leukemia stage.
Interactions between the intestinal microbiota and the mammalian host are essential for effective defense against pathogenic infection. However, despite critical associations between commensal bacteria and infection, the underlying mechanisms by which protective microbial cues are integrated by host cells remain unclear. Here, we find that the intestinal epithelial cell (IEC)-associated commensal bacteria, Segmented Filamentous Bacteria (SFB), enable early protection against Citrobacter rodentium that did not require CD4+ T cells. Expression of epigenetic modifiers in IECs enable microbiota to protect against C. rodentium infection, provoking the hypothesis that commensal bacteria may improve defense by instructing epigenetic modifications in IECs.

Consistent with this hypothesis, we found that SFB colonization induced enrichment of the enhancer-associated histone modification, H3K27Ac, within regulatory regions of genes that were significantly enriched for retinoic acid receptor (RAR) binding motifs. Many RAR-target genes were enriched in host defense pathways and exhibited increased expression during infection, suggesting that SFB may prime protection against infection through the RA pathway. Interestingly, SFB colonization increased RA levels in the intestine and supplementing RA to SFB-deficient mice decreased C. rodentium infection, whereas inhibiting RAR signaling in SFB-colonized mice increased pathogen burden. Collectively, these data suggest that primed RAR activation in IECs may represent a novel mechanism by which commensal bacteria calibrate defense in the intestine.
NF1 Patient Missense Variants Predict a Role for ATM in Modifying Neurofibroma Initiation

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In Neurofibromatosis type 1, NF1 gene mutations characterize benign plexiform neurofibroma (PNF) Schwann cells (SC), and no other genomic changes have been identified that explain patient-to-patient variability in tumor numbers. Evidence from twin studies suggests that NF1 variable expressivity is caused by unidentified modifier genes (Sabbagh et al., Hum Mol Genet., 2009). Whole exome sequencing data from SC and fibroblast DNA from the same resected PNFs confirmed SC biallelic NF1 mutations, and that non-NF1 somatic SC variants are variable, and present at low read number. We identified overrepresented germline variants, each present at low minor allele frequency in the general population, and many predicted as deleterious to protein function. Variants in these genes, including OBSCN, PKHD1, CUBN, CELSR2, COL14A1 and ATM, were also present in two additional, published, cohorts of NF1 patients (Gosline et al., Sci Data. 2017; Pemov et al., Oncogene, 2017). Many of these genes also showed decreased gene expression in neurofibromas versus human Schwann cells or nerves. Validating the relevance of identified variants, re-sequencing of tumor DNA confirmed ATM mutations. Also, ATM-relevant DNA repair defects were increased in the subset of neurofibromas with ATM variants, and in a subset of neurofibroma SC preparations and unselected tumors. In Nf1-/- mouse cells, genetic Atm loss promoted Schwann cell precursor self-renewal and increased tumor formation in Schwann cell precursor allografts, suggesting that reduced ATM expression can contribute to neurofibroma initiation. This was confirmed by intercrosses of ATM heterozygous mice with DhhCre; Nf1fl/fl neurofibroma-forming mice. Double mutants showed significantly increased numbers of plexiform neurofibromas. We conclude that ATM, and possibly other identified genes that show germline variants, are overrepresented in NF1 patients with neurofibromas, and are candidate modifiers of PNF pathogenesis. Supported by R37 NS083590 (Jacob Javits Merit Award) to NR.