Axel R. Concepcion, PhD– Feske lab

Functional genomics screens to define the ion channelome in T cells and adaptive immunity

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Ion channels and transporters (ICTs) are proteins that facilitate the movement of ions across cell membranes. Ionic signals are crucial to regulate many aspects of cell physiology. There are more than 600 ICTs that control ions transport across membranes but only ~10 ICTs are well established to play a functional role in T cells based on genetic evidence in mice and humans. Here we use: 1) transcriptomic analysis and 2) an unbiased in vivo functional genomics screen as independent and complementary approaches to identify novel ICTs that regulate T cell function. 1) Comparative transcriptome analysis of the ion channelome in CD4+ T cells and other cell types and tissues uncovered the specific subset of ICTs in T cells, of which only some have been shown to regulate T cell homeostasis and function. Gene knockdown of candidate ICTs in pathogenic Th17 cells demonstrates that their deletion protects mice from experimental autoimmune encephalomyelitis (EAE). 2) By using an unbiased and independent in vivo functional genomics screen with the lymphocytic choriomeningitis virus (LCMV) in CD4+ T cells, we found 10-12 novel ICTs that regulate T cell expansion and survival in antiviral immunity. Validation experiments of individual candidates in vitro and in vivo confirm the relevant role of these ICTs in clonal expansion of T cells after viral infection. Overall, our results demonstrate that functional genomics is a powerful approach to identify novel ICTs that regulate adaptive immune responses in vivo and thereby new therapeutic targets for the purpose of immunotherapy.

Christina Glytsou, PhD - Aifantis lab

Targeting mitochondrial structure sensitizes acute myeloid leukemia to Venetoclax treatment

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The BCL-2 family plays important roles in acute myeloid leukemia (AML). Venetoclax, a selective BCL-2 inhibitor, has received FDA approval for the treatment of AML. However, drug resistance ensues after prolonged treatment, highlighting the need for a greater understanding of the underlying mechanisms. Using a genome-wide CRISPR/Cas9 screen in human AML, we identified genes whose inactivation sensitizes AML blasts to Venetoclax. Genes involved in mitochondrial organization and function were significantly depleted throughout our screen, including the mitochondrial chaperonin CLPB. We demonstrated that CLPB is upregulated in human AML, it is further induced upon acquisition of Venetoclax resistance and its ablation sensitizes AML to Venetoclax. Mechanistically, CLPB maintains the mitochondrial cristae structure via its interaction with the cristae-shaping protein OPA1, whereas its loss promotes apoptosis by inducing cristae remodeling and mitochondrial stress responses.
Overall, our data suggest that targeting mitochondrial architecture may provide a promising approach to circumvent Venetoclax resistance.

Harold Elias, PhD - Park lab

Identification of Old HSCs with Preserved Self-Renewal and Long-Term Reconstitution Potential

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Aging hematopoiesis is characterized by increased numbers of immunophenotypic HSCs, but with impaired self-renewal and long-term reconstitution potential. We previously demonstrated that young mouse HSCs (CD34-CD150+LSK) can be fractionated into subsets based on expression of c-Kit surface expression, with c-Kithi HSCs exhibiting reduced self-renewal. We therefore hypothesized that the expansion of c-Kithi HSCs in old mice could potentially explain the age-related decline in immunophenotypically defined old HSC function. Evaluation of the bone marrow of 24- month-old C57Bl/6 mice revealed that the frequency of c-Kithi HSC (out of total HSCs) is 1.5-fold higher in old mice than in 3-month old mice (P=0.04). To test the long-term reconstitution potential of aging HSCs, we competitively transplanted c-Kitlo or c-Kithi HSCs from 24-month old mice, and observed that mice receiving old c-Kitlo HSC's exhibited significantly higher donor blood chimerism levels compared to old c-Kithi HSC recipients (57.1% vs 9.4%, P=0.02) and this was concordant with a 6.4-fold higher HSC chimerism achieved by old c-Kitlo HSCs. Mechanistically, preserved properties of stemness in c-Kitlo HSCs, was attributed to lower rates of global translation, as confirmed by OP-Puro assays.
Overall, our studies demonstrate a functional heterogeneity among old HSCs and identify a novel strategy to prospectively fractionate old HSCs to further investigate the molecular mechanisms of HSC aging

Sarah E. LeBoeuf, PhD – Papagiannakopoulos lab

Activation of oxidative stress response in cancer generates a druggable dependency on exogenous non-essential amino acids

Sarah E. LeBoeuf, Warren L. Wu, Triantafyllia R. Karakousi, Burcu Karadal, Kwok-kin Wong, Volkan I. Sayin, Thales Papagiannakopoulos

Rewiring of metabolic pathways is a hallmark of tumorigenesis as cancer cells acquire novel nutrient dependencies to support oncogenic growth. Identifying how oncogenic mutations in the context of the tumor microenvironment can drive unique nutrient requirements is crucial to uncover novel therapeutic strategies. Lung adenocarcinomas with KEAP1/NRF2-mutations, which activate the endogenous antioxidant response, can be therapeutically targeted by inhibiting glutaminolysis. Here we show that genetic, pharmacologic, and ROS-dependent activation of the NRF2 axis results in a dependency on multiple non-essential amino acids (NEAAs). This is mediated by the NRF2-dependent excretion of glutamate through the system xc- antiporter which limits availability of intracellular glutamate for NEAA synthesis. Dependency on NEAAs can be therapeutically targeted by dietary restriction or enzymatic depletion of individual amino acids. Importantly, low antioxidant tumors that lack alterations in the Keap1/Nrf2 pathway, such as Kras and p53 mutant tumors, which do not respond to known metabolic therapies, can be targeted by combination of glutaminase inhibition and dietary restriction of NEAAs.
Our findings identify a novel metabolic strategy to target cancers with activation of the Nrf2 antioxidant response pathway by restricting exogenous sources of NEAAs and these vulnerabilities can be exploited in a more general fashion in low antioxidant tumors.

Ioanna Tiniakou, PhD - Reizis lab

Genome-wide analysis of dendritic cell differentiation

Ioanna Tiniakou, Gorkem Garipler, Esteban O Mazzoni and Boris Reizis

Dendritic cells comprise genetically and functionally distinct subsets, including the interferon-producing plasmacytoid DCs (pDCs) and the antigen-presenting classical DCs (cDCs). In the steady state, DC development from common progenitors and subset specification in the bone marrow is driven primarily by the cytokine Flt3 ligand (Flt3L) and its receptor Flt3. It remains unclear how the same signaling pathway supports both progenitor proliferation and DC lineage commitment, necessitating a systematic search for molecular regulators of the process.
The conditionally immortalized progenitor cell line HoxB8-FL can be induced to differentiate into functional pDCs and cDCs, providing a unique tool to study Flt3L- driven DC development. We have retrofitted HoxB8-FL cells with Cas9 and used it to conduct a CRISPR-based knockout screen with a genome-wide sgRNA library.
Comparison of sgRNA content between undifferentiated progenitors and their DC progeny revealed highly significant differences, identifying candidate regulators of DC differentiation. Notably, sgRNAs targeting Pten were highly enriched in differentiated cells, suggesting that Pten deletion facilitates DC differentiation. These data are consistent with our previous identification of Pten as a negative regulator of cDC development.
Our data provide a proof of principle for the bona fide genome-wide genetic analysis of DC differentiation and suggest the utility of our system for the identification of novel
pathways controlling DC development.

Samik Upadhaya, PhD - Reizis lab

Visualization of endogenous hematopoietic stem cells in their native state

Samik Upadhaya, Oleg Krichevsky, David Fooksman, Boris Reizis

Hematopoietic stem cells (HSC) sustain lifelong hematopoiesis owing to their unique capacity for self-renewal and multilineage differentiation. In adult mammals, HSC reside in a specialized bone marrow (BM) niche which supports their maintenance and differentiation. HSC can be mobilized from their BM residence into peripheral blood, a procedure that can be utilized in clinical BM transplantation. However, the dynamics of endogenous HSC including their morphology and interactions with the BM niche has never been characterized in live animals. We adapted a transgenic mouse strain that allows specific fluorescent labeling of endogenous HSC to visualize them in their native environment using two-photon intravital microscopy. We found that HSC exhibit dynamic morphology with a majority of them undergoing short-range processive movements in the BM. The HSC were found to be in close proximity with stem cell factor (SCF)-expressing perivascular stromal cells. Following treatment with mobilization-inducing drugs, we observed a near-complete arrest of HSC movement.
Our results provide direct visual evidence of a highly dynamic nature of endogenous HSC and reveal their interaction with SCF-expressing stromal cells of the BM niche at
steady state.

Matthew Witkowski, PhD –Aifantis lab

The Evolving Immune Microenvironment of B-cell Acute Lymphoblastic Leukemia

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As with most cancer types, there remains a subset of B-cell acute lymphoblastic leukemia (B-ALL) patients who will relapse and succumb to therapy-resistant disease. In many cases, tumor heterogeneity underpins therapy failure leading to a leukemia- intrinsic model of clonal evolution, however, the bone marrow microenvironment most likely also plays a role in supporting leukemia survival, progression and escape from treatment. Here, we utilize single-cell RNA sequencing (scRNA-Seq) to generate a comprehensive map of the primary human B-ALL bone marrow immune microenvironment throughout three distinct stages of the disease: leukemia diagnosis, remission and relapse. These studies show extensive re-modeling of the immune microenvironment composition throughout the course of conventional chemotherapy and uncover a role for leukemia-associated non-classical monocytes in promoting B- ALL pathogenesis in vivo. Monocyte abundance at diagnosis is predictive of B-ALL patient outcome and, when targeted in Ph+ B-ALL animal models, leads to prolonged disease remission.

Our profiling of the human B-ALL bone marrow immune microenvironment provides a greater understanding of the potential extrinsic regulators of B-ALL survival and may highlight previously unknown environmental factors influencing immune-based treatment approaches to high-risk B-ALL.