21st Annual CAMB Symposium

Poster Abstracts

Odd #s: 10:20-11:25AM
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October 5, 2018
Perelman Quadrangle
University of Pennsylvania
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Arwa Abbas  

MVP  

Discovery of Novel Circular DNA Viruses through Metagenomic Data Mining  

Arwa Abbas, Louis Taylor, Frederic Bushman, Ronald Collman  

Viruses are highly abundant, but still largely uncharacterized. Studying viral communities is challenging because no common gene or sequence can be used to identify all viruses. The discovery and characterization of novel, highly divergent viruses with limited homology to database sequences is also difficult. Metagenomic techniques that use multiple displacement amplification of small amounts of viral nucleic acids are a powerful tool to identify novel viruses. We used this approach to initially isolate seven highly divergent circular DNA viral genomes from human bronchoalveolar lavage. Next, we developed a bioinformatics pipeline to query over 6000 samples from publicly available shotgun viral metagenomic datasets for homology to these novel sequences, ultimately recovering 19 additional complete genomes. These sequences primarily occur in human oral and respiratory samples and are notably absent in contamination controls and non-human samples. Viruses in this new group encode two proteins similar to capsid and replication-associated proteins from other small, circular, single-stranded DNA viruses, and one highly conserved open-reading frame without homology to any known protein family. Based on their striking dissimilarity to members in established genera and unique genomic architecture, these novel genomes likely constitute a separate family of eukaryotic viruses.
Mary Margaret Addison

MVP

Investigating the MHC Class II-Restricted Processing Landscape of HIV-1 Antigens

Mary M. Addison, Laurence Eisenlohr

Robust HIV-specific CD4+ T cell (TCD4+) responses are associated with decreased viral load and a slower progression to AIDS, and should therefore be considered in HIV vaccine development. TCD4+ are activated by antigen-derived peptides displayed in complex with MHCII molecules on the surface of antigen presenting cells (APC). According to the classical model, internalized antigens are catabolized and loaded onto MHCII in late endosomal compartments. However, alternative pathways, including endogenous processing, have been described. This occurs when virally-derived antigens synthesized within infected APCs are proteolyzed and loaded onto MHCII via a network of intracellular pathways. The relative contributions of the classical and alternative pathways to the HIV-specific TCD4+ response are unknown. Additionally, our understanding of the cell types that act as APCs during HIV-1 infection is incomplete. Notably, in addition to the “professional” APCs that present peptide via MHCII, TCD4+, which transiently express MHCII upon stimulation and are host cells for HIV, might act in this capacity. We have observed significant heterogeneity in the processing of HIV-1 proteins by dendritic cells and macrophages, and have shown that TCD4+ are able to present antigen from live virus. These findings could have significant implications for HIV-1 vaccine design.

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Jennifer Aleman

G&E

Nuclear pore component Megator directs nuclear distribution of a noncoding RNA and plays a role in dosage compensation

Jennifer Aleman, Maya Capelson

The Nuclear Pore Complex (NPC) is a large nuclear envelope-spanning protein complex, which consists of ~30 Nucleoporin components or Nups. Using Drosophila melanogaster as a model system, our lab has characterized an under-investigated nuclear structure formed by specific Nups of the NPC nuclear basket. We refer to this structure as the nuclear scaffold or nuclear cables. One of the main components of this structure is a nuclear basket Nup, Megator (Mtor), which has been functionally implicated in both transcriptional regulation and transport. To define the biological role of the Mtor-formed nuclear scaffold, we performed single molecule RNA FISH experiments targeting a non-coding RNA, roX1 in nuclei of Mtor-depleted and control salivary glands. roX1 is required for X chromosome dosage compensation. Our results from high-resolution imaging indicate that upon RNAi-mediated depletion of Mtor, roX1 is properly targeted to the X chromosome but is instead mis-localized in nuclear space. Upon further analysis of RNA levels by RT-qPCR, we show that roX1 transcript levels are increased. We also observe a male specific upregulation of X linked genes, linking Mtor to dosage compensation. Together, our results support a model, in which intranuclear filaments of Mtor function in restricting expression of dosage compensated genes.
Obesity and associated cardiovascular diseases including atherosclerosis cause morbidity and mortality worldwide. Recent research determining the mechanisms regulating thermogenic adipose tissue have generated potential therapeutics for obesity, but few of these targets have been harnessed to decrease cardiovascular risk. Perivascular adipose tissue (PVAT) surrounds blood vessels throughout the body and is uniquely poised to regulate atherosclerosis pathogenesis. Additionally, PVAT shares transcriptional and proteomic similarities to classical brown adipose tissue, suggesting factors that regulate thermogenic adipose may regulate perivascular adipose tissue. However, the role of the thermogenic program in regulating perivascular physiology is unknown. Here we report that thoracic PVAT increases thermogenic character following cold exposure. Moreover, we demonstrate that Early B Cell Factor 2 (Ebf2), a master regulator of brown adipocyte fate, expression in the vasculature is restricted to perivascular adipocytes. Deleting Ebf2 in adipocytes leads to loss of thermogenic gene expression in the thoracic and abdominal PVAT depots. We have generated a PVAT-selective Ebf2 knockout mouse utilizing the Sm22-Cre driver to specifically delete Ebf2 in perivascular adipocytes, sparing other major thermogenic depots. These mice will be used to determine if the thermogenic character of PVAT is required for acute cold responsiveness and atherosclerosis establishment and progression.
Polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) are a subset of MDSCs that are expanded and accumulated in several cancers. The main role of PMN-MDSCs in cancer is the suppression of anti-tumor immune responses. Tumor-associated PMN-MDSCs have potent immune suppressive effects compared to PMN-MDSCs in peripheral organs. However, the mechanism regulating the immune suppressive activity of tumor-associated PMN-MDSCs remain poorly understood. Recently, it was shown that splenic PMN-MDSCs from tumor-bearing mice treated with type 1 interferon (IFN1) have a decrease in their immune suppressive activity. It has been established that tumor-associated PMN-MDSCs are more immune suppressive, however, their response to IFN1 remains unclear. Therefore, our goal is to understand the role of IFN1 in the regulation of tumor-associated PMN-MDSCs function. We found that IFN1 receptor, IFNAR1, is downregulated in tumor-associated PMN-MDSCs compared to their counterparts in the spleen. To understand the importance of IFNAR1 signaling in PMN-MDSC biology, we utilized a knock-in mouse expressing a mutant form of IFNAR1 (Ifnar1SA) that is resistant to downregulation. Interestingly, PMN-MDSCs from Ifnar1SA mice were defective in their immune suppressive activity compared to PMN-MDSCs from WT mice. In this study, we described the possible role of IFN1 in negative regulation of PMN-MDSCs function in cancer.
Determining the role of human GBP1 in inflammasome activation during Legionella pneumophila infection

Antonia Bass, Sunny Shin

Host recognition of intracellular bacterial pathogens results in the formation of a multiprotein complex termed the inflammasome, which leads to proinflammatory cytokine secretion and pyroptosis. The caspase-1 dependent canonical inflammasome and the caspase-1 independent noncanonical inflammasome are both promoted by interferon receptor signaling. Guanylate binding proteins (GBPs) are interferon-inducible GTPases that promote inflammasome response to a variety of bacteria in mice. GBP functions in mice include bacteriolysis of cytosolic bacteria and rupture of pathogen-containing vacuoles in order to release pathogen-derived products into the cytosol, resulting in host recognition and inflammasome activation. Whether IFN-γ promotes inflammasome activation and upregulates GBPs in human macrophages is unknown. Here, we use Legionella pneumophila, an intracellular gram-negative bacteria, to study innate immune response. We hypothesize that human GBPs play a role similar to mouse GBPs through membrane rupture of the Legionella-containing vacuole to promote inflammasome response. We conducted IFN-γ priming experiments to look at whether IFN-γ promotes inflammasome response and upregulates GBP expression in primary human macrophages. Additionally, we employed siRNA-mediated knockdown of GBPs to determine whether they play a role in inflammasome response. Together, our results indicate that IFN-γ-induced human GBP1 is a key factor in inflammasome activation during Legionella infection.
Seasonal influenza viruses (IAV) are a major cause of human disease worldwide. Neutralizing antibodies (Abs) elicited by IAV primarily target the variable head domain of the hemagglutinin (HA) protein. Conventional influenza vaccines elicit Abs that primarily target the HA head-domain, and these vaccines must be updated each year to protect against antigenically drifted strains. Unlike the head domain, the HA stalk domain is highly conserved between different IAV strains. Although HA stalk-reactive Abs are subdominant, they can neutralize multiple different IAV strains in vivo. There is great interest in developing new universal immunization strategies that elicit broadly neutralizing Abs against conserved regions of HA, such as the stalk domain. HA stalk Ab-mediated protection has been demonstrated in vitro and in animals, but it is unknown if these Abs confer protection in humans. To determine the extent of protection mediated by stalk-specific Abs, we used human serological studies of community-acquired H1N1 infection to determine if HA stalk Abs are associated with protection from influenza infection. We found that serum levels of total IgG and IgA stalk antibodies were associated with protection from influenza virus infection in human cohort studies.
Anya Bauer

MVP

Identification of a novel genetic signature of enhanced viral kinetics in SHIV.C.CH505-infected rhesus macaques through limited passage

Anya Bauer, Hui Li, Emily Lindemuth, Shuyi Wang, George Shaw, Katharine Bar

Our lab developed a novel strategy to generate transmitted/founder (TF) chimeric simian-human immunodeficiency viruses (SHIVs) that encode native HIV-1 Envs and infect rhesus macaques (RM). Many TF SHIVs, however, are spontaneously controlled in a fraction of RM. We performed two sequential passage experiments using SHIV.C.CH505 with the goal of identifying the minimum number of mutations that confer increased viral fitness and preserved Env antigenicity. RM 5695 was inoculated with a mixture of plasma from 3 SHIV.C.CH505-infected RM. RM 5181 was inoculated with a mixture of plasma from RM 5695. Both RM 5695 and RM 5181 exhibited improved viral kinetics over baseline, with peak viremia of 106 copies/ml at 2 weeks post-infection (WPI) and set point viremia of 104-5 copies/ml through 24 WPI. Single genome sequencing identified a genetic signature of 6 mutations within Env, which evolved early and persisted in passage 1 and 2. SHIV.C.CH505 clones containing one or more selected mutations exhibited a viral fitness advantage in primary RM CD4 T cells. These results suggest that limited passage can enhance SHIV.C.CH505 viral kinetics and identify a minimally adapted clone that confers high peak and set point viremia.

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Bailey Baumann

**CPM**

**Cell-autonomous role of hepcidin in retinal iron homeostasis and under conditions of iron loading**

Bailey Baumann, Ying Song, Samira Littleton-Lakhal, Joshua L. Dunaief

Hepcidin (Hepc) regulates systemic iron levels by triggering the degradation of the iron exporter, ferroportin. Hepc is primarily produced by the liver, however, other organs, including the retina produce Hepc. Systemic HepcKO causes retinal iron overload. However, the role of retina-produced Hepc in regulating retinal iron levels is unknown. In this study, we determine whether liver or retinal Hepc controls retinal iron levels. We also investigate the retinal effects of long-term exposure to high iron levels. To investigate the role of Hepc in retinal iron regulation, we developed a liver-specific Hepc KO model, which has elevated systemic iron levels, and compared retinal iron levels in these mice to controls and systemic Hepc KO mice. We found that iron levels were elevated in both the conditional KO and systemic Hepc KO compared to controls, however, the largest increase in iron levels occurred in the RPE of conditional Kos. Elevated retinal iron levels in the conditional KO lead to the development of RPE degeneration and subsequent retinal dysfunction. These data suggest that blood iron levels are an important determinant of retinal iron levels. Gaining a better understanding of how changes in systemic iron levels affect retinal health has important clinical implications.
Gleb Bazilevsky

G&E

ATP-citrate lyase tetramerization is necessary for substrate binding and catalysis

Gleb Bazilevsky, Xuepeng Wei, Ronen Marmorstein

Acetyl-CoA is an essential metabolite required for lipogenesis, cholesterol synthesis, and epigenetic regulation. The study of acetyl-CoA metabolism can offer insight into glucose metabolism, cardiovascular health, and oncogenesis. In mammals, the majority of acetyl-CoA is produced by the essential enzyme ATP-citrate lyase (ACLY). ACLY is a large multidomain polypeptide that coordinates multiple cofactors to convert glucose-derived citrate into acetyl-CoA. However, there is little information about the mechanisms of ACLY regulation. Improved understanding of the mechanisms for ACLY function and regulation will elucidate the molecular underpinnings of efficient catalysis and provide targets for rational drug design. Previous observations suggest that the enzyme forms an evolutionarily-conserved multimeric complex with unknown significance for protein function. We hypothesize that ACLY multimerization is necessary for the full activity and stability of this enzyme. We used biochemical and biophysical approaches to determine that human ACLY is a homotetramer, maintained by the little-studied ACLY C-terminal citrate synthase homology domain. We present that the domain functions independently to coordinate tetramerization. Also, we show that the loss of this domain abolishes catalysis. Lastly, we demonstrate that tetramerization is necessary for cofactor binding. We propose that ACLY tetramerization is essential for activity by facilitating cooperative substrate binding.
Dana Bellissimo

*CB*

Runx1 loss increases inflammatory cytokine production by neutrophils

Dana Bellissimo, Mercy Gohil, Gerald Wertheim, Martha Jordan, D. Gary Gilliland, Nancy Speck

Mutations in RUNX1 (a hematopoietic transcription factor) occur frequently in acute myeloid leukemia but are not sufficient to promote leukemogenesis. ASXL1 (a polycomb group protein regulator) is the most frequently co-mutated gene with RUNX1. Given their significant co-occurrence, we sought to elucidate the mechanisms by which mutations in RUNX1 and ASXL1 cooperate in leukemia. We deleted Runx1 and Asxl1 alone and together in hematopoietic cells in mice using Vav1-Cre. Initially, double knock out (DKO) mice died of a fully penetrant disease; however, there was no evidence of myeloid malignancy. Subsequently, the lethal DKO phenotype markedly diminished after moving the mice to a different room, making malignancy an unlikely driver of the phenotype. Given the altered severity of the DKO phenotype after an environmental change, we explored the role inflammation may be playing. Phenotypically, DKO and Runx1 KO mice have decreased frequencies of eosinophils but normal frequencies of neutrophils and monocytes. Functionally, DKO and Runx1 KO neutrophils have increased TNF-alpha production in response to activation of Toll-like receptors 1/2, 4. This inflammatory phenotype was associated with increased NF-kappaB signaling. Future studies aim to elucidate the specific role of Runx1 in myelopoiesis as well as the mechanism underlying increased inflammatory responses.

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Elisabet Bjanes

MVP

Mitochondrial protein CARD19 regulates a cell death checkpoint downstream of caspase activation and Gasdermin D cleavage

Elisabet Bjanes, Kariana Rios, Alexandra DeLaney, Baofeng Hu, Naomi H. Philip, Dorothy Tovar, Brian C. Schaefer, Igor E. Brodsky

Distinct forms of cell death play a critical role in development, tissue homeostasis, and inflammatory responses to cellular stresses and microbial infections. During pyroptosis, Gasdermin D (GSDMD) is cleaved to enable IL-1 cytokine release and terminal cell lysis. Nevertheless, certain stimuli or cell types induce IL-1 cytokine release via formation of plasma membrane GSDMD pores without inducing cell death, suggesting that these two responses can be decoupled. How the final steps of cell death might be regulated downstream of GSDMD pore formation is unknown. Here we demonstrate that a mitochondrial-resident CARD-containing protein, CARD19, regulates terminal lysis downstream of multiple caspase-dependent death pathways, including pyroptosis. Despite being markedly protected from cell death, CARD19-deficient macrophages had no defect in caspase activation, secretion of IL-1 cytokines, or cleavage of GSDMD. Notably, CARD19-deficiency was associated with reduced levels of cleaved GSDMD into cellular membranes, and co-expression of CARD19 potentiated GSDMD-induced cell lysis. These findings genetically uncouple for the first time, cell death from caspase processing, IL-1 release, and GSDMD cleavage and demonstrate that CARD19 regulates a cell death checkpoint downstream of caspase-dependent GSDMD cleavage.

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Rachel Blomberg

GTV

Fibroblast activation protein confers resistance to obesity by restraining adipogenic differentiation and regulating matrix-mediated mTOR signaling

Rachel Blomberg, Daniel Beiting, Ellen Pure

Obesity is a risk factor for multiple diseases, including diabetes, cardiovascular disease, and cancer. Within obese adipose tissue, multiple factors contribute to creating a disease-promoting environment, including metabolic dysfunction, inflammation, and fibrosis. Recent evidence points to fibrotic responses, particularly extracellular matrix remodeling, in playing a highly functional role in the pathogenesis of obesity. Fibroblast activation protein plays an essential role in remodeling collagen-rich matrices in the context of fibrosis and cancer. We observed that FAP-null mice have increased weight compared to wild-type controls, and so investigated the role of FAP in regulating diet-induced obesity. Using genetically engineered mouse models and in-vitro cell-derived matrices, we demonstrate that FAP expression by preadipocytes restrains adipogenic differentiation. FAP-mediated matrix remodeling also alters lipid metabolism in part by regulating mTOR signaling. Together, via these mechanisms, FAP confers resistance to diet-induced obesity. The critical role of ECM remodeling in regulating obesity offers new potential targets for therapy.
Familial platelet disorder (FPD) is a genetic disease resulting in low platelet counts and increased bleeding. Some patients have germline mono-allelic mutations in either ETV6 or RUNX1 and a heightened risk of developing acute myeloid leukemia (AML), suggesting a common pathway may underlie both phenotypes. Mouse models have been used to study FPD/AML but none fully recapitulate the human phenotype. Alternatively, the directed differentiation of iPSCs can be used to elucidate the mechanism of FPD/AML, and determine if these transcription factors function in common pathways. We have genome edited a wild type iPSC line via CRISPR/Cas9 to generate isogenic iPSC lines harboring mono-allelic mutations in ETV6 (ETV6+/mut) or RUNX1 (RUNX1+/mut) line). We also generated an iPSC line with a homozygous ETV6 mutation (ETV6mut/mut). The RUNX1+/mut line generates fewer hematopoietic progenitors, whereas both mutant ETV6 iPSC lines generate more. Upon megakaryocyte differentiation, ETV6+/mut and ETV6mut/mut iPSC lines generate more megakaryocytes but they are less functional as seen by lower expression of mature megakaryocyte markers as well as poor activation upon stimulation. Conversely, RUNX1+/mut iPSCs generate fewer but more mature megakaryocytes with enhanced responsiveness to stimulation. Preliminary studies show enhanced cytokine production in some myeloid subpopulations upon LPS stimulation in both mutant lines.
Niambi Brewer

G&E

Gnas inactivation alters adipose tissue properties during progression to heterotopic ossification

Niambi Brewer, John T. Fong, Deyu Zhang, Frederick S. Kaplan, Robert J. Pignolo, Eileen M. Shore

Heterotopic ossification (HO) is a common physiological response to severe tissue trauma such as combat blast injuries, high impact trauma, and hip replacements. HO in the rare genetic disorder, Progressive Osseous Heteroplasia (POH), initiates in subcutaneous soft tissues then progresses into deeper connective tissues. POH is caused by inactivation of GNAS. To examine the mechanisms and signals that lead to initiation of HO via GNAS inactivation, we developed an in vivo HO model using Gnas-null mice (Gnasfl/fl;Cre-ERT2 or Gnasfl/fl;Ai9fl/fl;Cre-ERT2) that consistently and reliably induces spontaneous HO. Through microCT, histologic, and immunohistochemistry analyses in cross-sectional and longitudinal studies, this model initiates HO within subcutaneous adipose tissues with progressive expansion over time. Using the same model and methods, we identified increased extracellular matrix content and changes in adipose tissue composition preceding HO formation. Cell implant studies showed that fluorescent-labeled Gnas-null ASCs form HO when implanted within Gnas-null adipose tissue. However, wildtype (WT) ASCs also induce HO in a Gnas-null background, although less robustly than mutant ASCs, suggesting that the tissue microenvironment strongly influences cell fate. Further, neither WT nor Gnas-null ASCs implanted into a control background formed HO, supporting that the mutant osteogenic progenitor cells are insufficient to induce HO.

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Rebekah Brooks

CB

A circadian-oscillating long non-coding RNA could negatively regulate the PBAF complex to promote cell growth in ccRCC

Rebekah C. Brooks, Zandra E. Walton, Lin Zhang, Chi V. Dang

Our lab found that acidosis commonly found in the tumor microenvironment dampens the circadian transcriptome by downregulating the translation of a key component of the molecular clock, BMAL1. Surprisingly, some genes maintain rhythmicity at low pH despite the decrease in BMAL1 expression. ADIRF-AS1, a long non-coding RNA (lncRNA) of unknown function, persistently oscillates at low pH in a BMAL1 dependent manner. Loss of ADIRF-AS1 leads to decreased proliferation in a U2OS osteosarcoma cell line, prompting us to evaluate cis and trans functions. While neighboring genes were unperturbed by the loss of ADIRF-AS1, an RNA pulldown assay followed by proteomics analysis suggested a direct binding interaction between ADIRF-AS1 and the polybromo-associated BAF (PBAF) chromatin modifying complex. PBAF regulates cell growth and metabolism. PBAF complex proteins are mutated in approximately 40% of clear cell renal carcinomas (ccRCC). According to TCGA data for ccRCC, ADIRF-AS1 expression is elevated and higher expression correlates with decreased survival. Further, gain of function experiments determined that overexpression of ADIRF-AS1 leads to increased proliferation in ccRCC cell lines that express wild-type PBAF components. These data reveal the regulation and potential function of a previously uncharacterized lncRNA, ADIRF-AS1 that could impair the PBAF complex in ccRCC.
Blake Caldwell

G&E

TET2-mediated passive DNA demethylation antagonizes iPSC generation in a MEF reprogramming model

Blake Caldwell, Rahul Kohli, Marisa Bartolomei

In cell culture, somatic differentiation can be reversed through the overexpression of Oct4, Sox2, and Klf4 (OSK) to generate induced pluripotent stem cells (iPSCs). This process is dependent on enzymes of the Ten-eleven Translocation (TET) family, which promote active DNA demethylation. Active DNA demethylation can proceed through two distinct pathways: active modification with passive dilution (AM-PD) or active modification with active removal (AM-AR). Recent evidence suggests that AM-PD demethylation is insufficient for iPSC reprogramming, indicating the two DNA demethylation pathways are not functionally equivalent. In order to test the influence of different modes of DNA demethylation on iPSC reprogramming, we performed OSK induction on MEFs overexpressing wild-type Tet2WT, catalytically inactive Tet2HxD, or hypomorphic Tet2T1285E, which is competent for AM-PD but not AM-AR demethylation. Surprisingly, overexpression of any of the TET2 variants led to diminished epithelial gene activation, a critical step in early MEF reprogramming. However, 10 days post-OSK induction, only Tet2T1285E overexpression was observed to significantly decrease the observed number of pluripotent colonies relative to OSK treatment alone. These experiments suggest that TET2’s AM-PD activity has a dominant negative effect on reprogramming efficiency, and represent the first evidence of biological activity specifically attributable to one mode of DNA demethylation.

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Intermediate C9orf72 repeat expansions in Corticobasal Degeneration

Christopher Cali, Maribel Patino, Jessica Phan, Edward B. Lee

Large expansions in C9orf72 (100s-1000s) are the most common genetic cause of Amyotrophic Lateral Sclerosis (ALS) and Frontal Temporal Degeneration (FTD). However, whether intermediate expansions also contribute to neurodegenerative disease is not well understood. Several studies have identified intermediate repeats in Parkinson’s disease patients, but the association was not found in autopsy confirmed cases. We instead hypothesized that intermediate C9orf72 repeats are a genetic risk factor for Corticobasal Degeneration (CBD), a neurodegenerative disease that is clinically similar to Parkinson’s, but can only be confirmed at autopsy. Indeed, intermediate (>17) C9orf72 repeats were significantly enriched in autopsy-proven CBD (n=355 cases, odds ratio=2.12, p-value=0.0094). In contrast to cases of FTD/ALS with large C9orf72 expansions, CBD with intermediate C9orf72 repeats was not associated with pathologic RNA foci or dipeptide repeat protein aggregates. While large C9orf72 repeat expansions are known to decrease C9orf72 expression, intermediate C9orf72 repeats result in increased C9orf72 expression in human brain tissue and CRISPR/cas9 knockin cells. Finally, overexpression of C9orf72 in HEK293T Tau biosensor cells results in increased tau aggregation, thereby implicating C9orf72 directly with CBD pathogenesis. These results indicate that therapies that reduce C9orf72 expression may be beneficial for the treatment of tauopathies such as CBD.
Repulsive and attractive guidance receptors, Robo and Fra respectively, function in guidance by inducing local cytoskeletal changes in axons. Although some downstream effectors of these guidance receptors have been identified, the exact links to the cytoskeleton and the nature of these cytoskeletal changes are still unclear. Recent studies have implicated the heteropentameric Scar/Wave Complex (SWC), a complex involved in actin polymerization, in axon guidance. The cytoplasmic domains of both Robo and Fra have the conserved binding sequence (the WIRS motif) for the Scar/Wave Complex. Drosophila mutants of the complex show phenotypes similar to robo1 mutants. This suggests that the complex might function as an effector of Robo and Fra, and serve as a link to the cytoskeleton. Preliminary results show that mutants of members of the Scar/Wave complex enhance crossing defects seen in the ventral nerve cords of Drosophila embryos with reduced Robo or Fra function. Mutating the WIRS binding site for the Scar/Wave complex in Robo and Fra reduces receptor activity in gain-of-function assays in the Drosophila embryonic nerve cord. Identifying downstream effectors of guidance receptors that can modulate the cytoskeleton will provide insight into how these receptors function in neural circuit formation and wiring specificity.
Role of the hexosamine biosynthesis pathway in pancreatic cancer

Sydney L. Campbell, Clementina Mesaros, Tiffany Tsang, Luke Izzo, Ian A. Blair, Kathryn E. Wellen

The hexosamine biosynthesis pathway (HBP) takes inputs from glucose, glutamine, acetyl-CoA, ATP, and uridine diphosphate to produce uridine diphosphate N-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is the major substrate for synthesis of sugars used in N- and O-linked glycosylation, as well as O-GlcNAcylation in the cytosol and nucleus. Interestingly, the HBP is upregulated in pancreatic cancer, though its regulation and role in cancer progression are unknown. Our data shows that UDP-GlcNAc levels are robustly maintained by PDAC cells across a range of stress conditions, and that PDAC cells show dynamic flexibility in synthesis of UDP-GlcNAc to achieve this. We also show that disruption of the HBP results in impaired proliferation across PDAC cell lines. Thus, we hypothesize that the HBP is critical for PDAC cell proliferation and with this project aim to determine the mechanism linking the HBP and growth.
Tcf-1-related transcriptional network drives a binary effector versus exhaustion CD8+ T cell fate decision


During chronic infection and cancer, the terminal effector CD8+ T cells disappear and CD8+ T cells differentiate into PD-1+ exhausted T cells (TEX) with poor effector function. It is now clear that TEX represents a distinct CD8+ T cell fate. However, the underlying fate decision events between terminal effector (TE) and TEX remain poorly understood. Here we defined a binary fate change early during the development of CD8+ T cell exhaustion where TE formation and generation TEX precursor opposing neutrally exclusive fate decision. Moreover, the TE fate is driven to excessive activation and rapidly depleted during chronic infection, resulting in a protective immunity lesion that allows pathogen persistence, but also protected of immune pathology. In contrast, TEX precursors are KLRG1-PD-1+ and express Tcf-1. Single cell RNA-sequencing on antigen specific CD8+ T cells at early chronic infection stage defined an effector biased transcription factor (TF) cluster and a TEP biased cluster centralized by Tcf-1. Genetic depletion of Tcf-1 skews TEX cell fate to chronic terminal effectors, and PD-1 helps to stabilize the TEP cell pool. Combination of epigenetic analysis and genetic manipulation confirms that Tcf-1 regulates multiple molecular modules of durable TEX cells through several TFs. Tcf-1 is important to mediate T-bet/Eomes transition by enhancing Eomes expression; Tcf-1 also keeps high expression of c-Myb-Bcl-2 axis for the persistence of TEX population. This study addresses the evolutionary significance for antigen specific T cell going into exhaustion program for long-term antigen control and keeps homeostasis of the host-pathogen balance. Moreover, this study also uncovers how Tcf-1 maintaining the durable TEX population through multiple downstream TFs.

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Regulatory Network Topology Affects the Frequency of Spontaneous Polymyxin Resistance Among Enterobacteriaceae

Annie Chen, Alex Seidel, Jay Zhu, Mark Goulian

Polymyxins have emerged as last-resort antibiotics for many multidrug-resistant Proteobacteria. These cationic antimicrobial peptides are thought to disrupt the outer membrane of Gram-negative bacteria through interactions with negatively charged lipopolysaccharides (LPS). Curiously, the frequency of spontaneous polymyxin resistance is much higher in Klebsiella pneumoniae compared to other genera of Enterobacteriaceae. The PmrB/PmrA two-component system mediates polymyxin resistance by regulating expression of LPS modification enzymes that decrease the negative charge of the outer membrane. The phosphorylation state of the response regulator PmrA is controlled by its cognate sensor kinase PmrB and also by the noncognate sensor PhoQ via the connector protein PmrD. Based on analysis of polymyxin-resistant isolates, the most common mechanism of resistance in K. pneumoniae is inactivation of the gene mgrB, which encodes a negative regulator of PhoQ. In contrast, we found that in standard resistance assays, deletion of mgrB only confers a small level of protection in Salmonella enterica and no protection in Escherichia coli. We show that these phenotypic differences can be explained by topological differences in the polymyxin resistance network. We further show that PhoQ/PhoP can activate PmrB/PmrA in E. coli in certain growth conditions, indicating that environmental stimuli affect the connectivity of this network.
Ryan Cheng
DSRB

The role of guidance receptors nrp2a and nrp2b in protoglomerular targeting of OMP and TRPC class olfactory sensory neurons

Ryan Cheng, Puneet Dang, Yoon-Ji Moon, Zhili He, Jonathan Raper

The targeting of olfactory sensory neuron (OSN) axons as they project from the olfactory epithelium to the olfactory bulb is a good system in which to study the development of complex, stereotyped neuronal circuitry. Two classes of OSNs express either OMP or TRPC in the zebrafish. These two populations of neurons target distinct, stereotyped sets of protoglomeruli in the olfactory bulb. Using RNAseq, we identified the axon guidance factors nrp2a and nrp2b, and their potential ligand sema3fa as candidate guidance factors that are differentially expressed within these two classes of sensory neurons. To investigate their role in protoglomerular targeting, mutant lines were generated and the targeting fidelity of OSNs labeled by OMP:RFP and TRPC:Venus was assessed. We find that in nrp2a, nrp2b, or sema3fa mutants, TRPC expressing OSNs misproject into OMP specific glomeruli. Misprojection phenotypes in nrp2a;nrp2b double mutants suggest a synergistic genetic interaction between them. Through in vitro binding assays we show that sema3fa is a potential ligand for nrp2a and nrp2b. Our results suggest that nrp2a and nrp2b act synergistically in the protoglomerular targeting of TRPC expressing OSNs and that sema3fa is likely one of a few ligands involved in OSN axon guidance mediated through the nrp2s.

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TAM receptors attenuate NK cell responses via E3 ubiquitin ligase Cbl-b

Leilani M. Chirino, Suresh Kumar, David Sterner, Mariko Okumura, Taku Kambayashi

TAM receptors (Tyro3, Axl, and Mer) are receptor tyrosine kinases that inhibit pro-inflammatory responses in a variety of innate immune cells including natural killer (NK) cells. TAM receptor ligation blocks NK cell activation and the inhibition of TAM receptor signaling prevents metastatic progression in a mouse tumor model in an NK-cell dependent manner. Despite the importance of TAM receptors, the mechanism by which they negatively regulate NK cells is unknown. We hereby show that Tyro3 activates the E3 ubiquitin ligase Cbl-b by phosphorylating its tyrosine residues 133 and 363. Cbl-b inhibits activating receptor signaling in NK cells by ubiquitination and subsequent degradation of key signaling molecules. Thus, we hypothesized that TAM receptors may inhibit NK cell function by activating Cbl-b. Indeed, the ligation of TAM receptors by their ligand Gas6 suppressed activating receptor-stimulated NK cell function (IFNγ production and degranulation) in a TAM receptor kinase- and Cbl-b-dependent manner. Moreover, Gas6 ligation induced the degradation of LAT1, a transmembrane adaptor protein required for NK cell activating receptor signaling, in WT but not in Cbl-b KO NK cells. Together, these results support a novel mechanistic model by which TAM receptors negatively regulate NK cell function through phosphorylation and subsequent activation of Cbl-b.
A C. elegans Patched-related protein is required for apical extracellular matrix assembly of a Zona Pellucida domain protein

Jennifer Cohen, Meera Sundaram

Apical extracellular matrices (aECMs) shape and protect apical surfaces, like tube lumens and the epidermis. aECMs are rich in Zona Pellucida domain (ZP) proteins, whose dysfunction is associated with human diseases. However, how ZP proteins assemble in the aECM is unclear. We are using one C. elegans ZP protein, LET-653, to study how ZP proteins incorporate into the aECM. To define LET-653 aECM localization and function, we tagged endogenous LET-653 with CRISPR and performed structure-function analysis. LET-653::SuperfolderGFP (SfGFP) was present in two distinct aECM layers – along the apical membrane, and in a fibrous luminal core. Furthermore, LET-653’s ZP domain C-terminus, the ZPc subdomain, was necessary and sufficient for LET-653’s apical membrane localization and to rescue let-653 mutants. Via RNAi screening for genes that promote LET-653 membrane localization, we identified the patched-related gene ptr-4. Patched-related genes encode multi-pass transmembrane proteins with proposed roles in trafficking and signaling. We determined that PTR-4 lined external epithelial apical surfaces using CRISPR-generated PTR-4::SfGFP. Additionally, ptr-4 mutant phenotypes mimicked those of aECM mutants, indicating that ptr-4 may promote aECM function. Together, these data support a model in which a patched-related protein promotes matrix assembly by anchoring a ZP protein to the apical membrane.
Disrupted calcium signaling at endoplasmic reticulum-mitochondria contact sites promotes neuroblastoma multidrug resistance

Jorida Coku, David M. Booth, Madison C. Pedrotty, Jamie C. Ye, Annette Vu, Kangning Liu, C. Patrick Reynolds, György Hajnóczky, Michael D. Hogarty

Many neuroblastoma patients succumb to multidrug resistant disease. Resistance is attributed to insensitivity to stress-induced apoptosis, however, its mechanisms remain obscure. Mitochondria (mito) provide platforms for integrating stress signals to determine cell survival or death. Mito interact with endoplasmic reticulum (ER) at contact sites that regulate calcium and lipid transfer to modulate apoptotic sensitivity. These ubiquitous ER-mito contacts are maintained by tethering complexes that include MFN2 and PACS2. We studied neuroblastomas from diagnosis (DX) and relapse (REL) from the same patients treated with high-risk therapy. Mitochondria from REL tumors resist apoptosis induction when directly exposed to death effectors tBid and BimBH3 using mitochondrial profiling in 7 of 7 tumor pairs, providing a functional biomarker that correlates with multidrug resistance. EM imaging revealed reduced ER-mito contact number and gap-distance in REL cells as confirmed by IB for organelle-specific proteins. Disruption of ER-mito contacts by MFN2 or PACS2 shRNA attenuated mitochondrial responses and phenocopied resistance. ER-derived calcium transfer measured by cytosolic and mito calcium reporters was decreased in REL cells. Increasing ER-mito connectivity via synthetic linkers improved calcium transfer in REL cells. We demonstrate that altered calcium signaling at ER-mito contact sites plays a principal role in our therapy resistance phenotype.
Courtney Comar

MVP

**NS4a antagonizes PKR activation and Type III interferon expression during MERS coronavirus infection**

Courtney Comar, Stephen Goldstein, Yize Li, Boyd Yount, Ralph Baric, Susan Weiss

In 2012, a novel coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), emerged from dromedary camels into humans and has caused outbreaks of severe respiratory illness. Type I and III interferons are among the earliest responses to viral infection. Sensing of viral dsRNA by RIG-I-like receptors initiates a signaling cascade that induces antiviral interferon (IFN) production. Coronaviruses induce a delayed and relatively weak interferon response, most likely due to their antagonism of IFN and other antiviral dsRNA-induced pathways. The functions of MERS-CoV accessory proteins are mostly uncharacterized, however previous overexpression reporter system assays suggest that MERS-CoV NS4a binds dsRNA, acts as a potent IFN antagonist, and blocks PKR activation. However these studies fail to characterize the localization or function of NS4a during MERS-CoV infection. We used a recombinant MERS-CoV mutant that does not express NS4a to examine the interactions of NS4a with the innate immune system. During infection with this mutant, expression of a type III IFN and some interferon stimulated genes is increased compared to infection with wild type. We found that PKR is phosphorylated during NS4a mutant infections. The modest effects we see indicate that other viral proteins or mechanisms are blocking activation of these pathways.
Lisa Cucolo, Jingya Qiu, Martha Jordan, Andy Minn

Cancer immunotherapies such as CTLA4 or PD1/PDL1 immune checkpoint blockade (ICB) can result in impressive clinical responses of multiple cancer types, however, only a minority of patients respond. One factor that contributes to tumor resistance to ICB is an unfavorable tumor immune microenvironment. How cancer cells influence the accumulation of immune suppressive populations is poorly understood. We discovered that diminished expression of the Receptor Interacting Protein Kinase 1 (RIPK1) pathway occurs in ICB responsive murine tumor models. RIPK1 is a critical regulator of inflammation and cell death. It orchestrates responses to death receptors, pattern recognition receptors, and inflammatory cytokines, leading to either cytokine release and pro-survival signals or cell death. To test the contribution of RIPK1 to ICB efficacy and whether this cytokine signaling hub can impact the immune landscape of tumors, we genetically deleted RIPK1 in two murine tumor cell lines. This results in improved overall survival and tumor control upon ICB treatment. Deletion of RIPK1 in the tumor cells also resulted in changes in inflammatory cytokine secretion and corresponding favorable changes in immune infiltrate, suggesting that RIPK1 is a potential target for combinatorial therapies to improve patient response to ICB.

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Sera from individuals with narrowly focused influenza virus antibodies rapidly select viral escape mutations in ovo

Amy Davis, Kevin McCormick, Megan Gumina, Joshua Petrie, Emily Martin, Katherine Xue, Jesse Bloom, Arnold Monto, Frederic Bushman, Scott Hensley

Influenza viruses use distinct antibody escape mechanisms depending on the overall complexity of the antibody response that is encountered. When grown in the presence of a hemagglutinin (HA) monoclonal antibody, influenza viruses typically acquire a single HA mutation that reduces the binding of that specific monoclonal antibody. In contrast, when confronted with mixtures of HA monoclonal antibodies or polyclonal sera that have antibodies that bind several HA epitopes, influenza viruses acquire mutations that increase HA binding to host cells. Here, we completed a series of experiments to determine if humans with narrowly focused HA antibody responses are able to select for influenza virus antigenic escape variants in ovo. We identified three human donors that possessed HA antibody responses that were heavily focused on a single HA antigenic site. Remarkably, sera from all three of these donors selected single HA escape mutations during in ovo passage experiments, similar to what has been previously reported for single monoclonal antibodies. These single HA mutations directly reduced binding of serum antibodies used for selection. We propose that new antigenic variants of influenza viruses might originate in individuals that produce antibodies that are narrowly focused on HA epitopes.
Caspase-8 regulates IL-12p40 expression through c-Rel translocation

Alexandra DeLaney, Corbett Berry, Andrew Hart, Igor Brodsky

Host cells strike a balance between activation and tolerance in response to inflammatory or infectious stimuli to induce acute responses to infection while preventing chronic inflammation. Understanding the mechanisms by which host cells regulate production of inflammatory mediators and appropriately define the balance between inflammation and tolerance remains an outstanding question in immunology and cellular biology. A protein at the center of this interface is the cysteine protease caspase-8. Caspase-8 plays an evolutionarily conserved role in activation of cell-extrinsic apoptosis downstream of TNF receptor (TNFR) and Toll-Like Receptor (TLR) signaling. However, in addition to these well-established functions of caspase-8, we and others have found that caspase-8 plays a cell-intrinsic role in inflammatory gene expression in response to multiple TLR agonists. We have found that caspase-8 deficiency is correlated with decreased expression of key inflammatory mediators, including IL-12, TNF, IL-1β, IL-1α, in response to TLR stimulation. We find that caspase-8 deficiency is associated with decreased phosphorylation of the Inhibitor of IκB Kinase (IKK) complex, as well as decreased nuclear translocation of c-Rel. c-Rel is a key regulator of TLR-induced IL-12 expression. We therefore propose that caspase-8 regulates a subset of inflammatory genes via control of cRel translocation.
Erica Dhuey

CB

The Role of the T cell Repertoire in Response to Immune Checkpoint Blockade for Cancer

Erica Dhuey, Olivia Oldridge, Roshan Ravishankar, Andy Minn

Immune checkpoint blockade (ICB) represents a paradigm shift in cancer treatment. ICB is effective against several cancer types, including particularly aggressive cancers such as metastatic melanoma. Remarkably, some patients treated with ICB experience durable responses previously unseen in metastatic disease. However, the majority of patients do not benefit from therapy, necessitating a deeper understanding of the molecular determinants of response. Some clinical studies have established a positive correlation between tumor-specific “neo-antigen” (Ag) burden and response to ICB therapy, suggesting that ICB response is limited by the ability of T cells to recognize tumors. However, these studies also suggest that patients with low neo-Ag load may not benefit from ICB therapy, necessitating sensitizing therapies. We find that treatment with a receptor activator of nuclear factor kappa-B ligand (RANKL) blocking antibody, which inhibits central tolerance leading to the release of self-reactive T cells, reduces tumor burden in models with limited neo-Ag repertoires. Furthermore, RANKL blockade sensitizes these tumors to ICB therapy, resulting in complete remissions. We demonstrate that this effect is T cell-mediated, and causes an increase in self-Ag specific T cell infiltration and proliferation. These studies provide key insight into the role of the T cell repertoire in cancer immunotherapy.

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Leela Dilley

G&E

The genetic control of sleep ontogeny in Drosophila

Leela C. Dilley, Milan Szuperak, Charlette E. Williams, David S. Garbe, Matthew S. Kayser

Nearly all species exhibit sleep ontogeny, including increased sleep amount in early life, which is thought to facilitate brain maturation. However, knowledge of the genetic factors controlling early life sleep is lacking. Sleep in Drosophila shares many features with mammalian sleep, including increased sleep time and intensity in early life. Here, we investigate the genetic control of sleep ontogeny. We find that ontogenetic changes in sleep persist in all studied Drosophila sleep mutants, suggesting that the genes regulating sleep ontogeny are distinct from those regulating mature adult sleep. To identify sleep ontogeny-specific genes, we conducted a RNAi-based screen, defining “hits” as lines lacking increased sleep amount in young adulthood. Follow up approaches converged on the transcription factor PDM3 as a novel genetic regulator of sleep ontogeny. Temporal mapping experiments show that PDM3 acts during early development to control sleep ontogeny. Our previous work showed that sleep ontogenetic change derives from developmentally-regulated activity changes in the fly central complex. Interestingly, loss of PDM3 disrupts innervation of the central complex, suggesting PDM3 acts during development to control the patterning of sleep circuits. Dissecting the genetic control of sleep ontogeny will provide a platform for investigating behavioral sequelae of developmental sleep abnormalities.

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Jennifer Dumaine

MVP

Cryptosporidium parvum exports proteins into the cytoplasm of the epithelial host cell

Jennifer Dumaine, Adam Sateriale, Amita Reddy, Boris Striepen

Infection with the protozoan parasite Cryptosporidium is a leading cause of diarrheal disease and child mortality worldwide. Cryptosporidiosis is typically self-limiting, but in the context of malnourishment or immunodeficiency the disease can be protracted and deadly. Currently, there are no vaccines and only a single drug of limited efficacy. Upon infection, Cryptosporidium travels to the small intestine, where it establishes an intracellular but extracytoplasmatic parasitophorous vacuole leading to dramatic remodeling of the epithelial cell cytoskeleton. We hypothesize that effector proteins exported into the host cell play critical roles in establishment and maintenance of infection by modulating interaction with host immunity. We assembled a prioritized list of candidate effectors based on a variety functional genomic and population genetic parameters. Using the CRISPR/Cas9 system to epitope tag the endogenous loci of candidate proteins, we have identified Medle 2 as the first example of a host targeted protein in Cryptosporidium parvum. Medle 2 is highly polymorphic and localizes to the cytoplasm of infected HCT-8 cells in tissue culture, and to the cytoplasm of intestinal epithelial cells in infected mice. Our current work aims to uncover the function of Medle 2 during C. parvum infection through cell biological, transcriptional and functional proteomic studies.

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Targeting Melanoma with CAR-T Therapy

Kelly Lynn Dunlevy, Nicholas Minutolo, Christoph Ellebrecht, Aimee Payne, Daniel J. Powell, Todd W. Ridky

Melanoma is the deadliest form of skin cancer, with a 5-year survival rate of approximately 30% for advanced disease. Although advanced disease often initially responds to targeted and immune therapeutics, tumor resistance is common. Chimeric antigen receptor (CAR) T-cells have shown great promise in treatment of leukemia and lymphoma but remain challenging to target against solid tumors due to the difficulty of finding tumor specific antigens to reduce on-target, off tumor toxicity. In order to target melanoma with CAR T cells with limited on-target, off tumor toxicity, we designed CAR constructs against MC1R, a G-coupled protein receptor with expression restricted to melanocytes and melanoma, utilizing mutated agouti (AgF117A), an endogenous ligand of MC1R, in the ectodomain. This mutant preferentially binds MC1R over other MCR proteins. Preliminary data using AgF117A CAR in vitro demonstrate toxicity specific towards MC1R expressing primary human melanocytes and WM46 cells (a BRAF V600E driven melanoma cell line) with limited toxicity towards human fibroblasts. In vivo, a single infusion of AgF117A CAR T-cells temporarily regressed tumors and extended survival, with limited toxicity in a subcutaneous mouse model of BRAF V600E melanoma. Taken together, these data suggest MC1R is a suitable target for CAR-T cell therapy in melanoma.
Louis Taylor

*MVP*

Uncovering catalytic activities of the replication-associated protein from a new group of small DNA viruses

Louis Taylor, Arwa Abbas, Young Hwang, Ronald Collman, Frederic Bushman

Viruses are highly abundant and important in human health, but most species remain uncharacterized. We identified eighteen members of a new family of divergent small, circular DNA viruses through metagenomic sequencing of human respiratory samples. Here, we dissect the catalytic functions of the replication-associated (Rep) protein to understand the replication mechanism of these novel viruses. Rep proteins catalyze rolling-circle replication in many small, circular DNA viruses and plasmids. To test the capability of the Rep protein to catalyze reactions important for genome replication, we developed multiple in vitro biochemical assays. We show that Rep catalyzes two distinct reactions on a supercoiled, dsDNA substrate: 1) nicking, a fast reaction which probably occurs early in genome replication, and 2) joining, a slow reaction likely involved in resolution of unit genomes. Rep-mediated strand cleavage is sequence- and strand-specific. Finally, Rep activity on a relaxed, double-stranded circular DNA (most similar to the native viral genome) requires Mg+2 and ATP and is abolished after mutation of key, conserved catalytic and ATP-dependent helicase residues. These data provide insight into the replication mechanisms of this new group, important for better understanding their biology, and could provide a foundation for establishing a cell culture system.

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Monika Eiva

Preservation of immunity by self-renewing stem cell memory T cells in human cancer

Monika A. Eiva, Daniel J. Powell Jr.

Despite advances in chemotherapy and surgery, ovarian cancer (OC) is the most lethal gynecological cancer in women. Developing effective immunotherapies that generate long-lasting anti-tumor responses could greatly improve OC patient outcomes. The presence of tumor infiltrating lymphocytes (TILs) is a positive prognostic factor for OC, implicating an active role for TILs in the control of OC progression, and foretelling that at least a subset of these T cells has the capacity for autologous tumor cell killing. The Powell lab first identified CD137 as a biomarker for a subset of naturally-occurring “tumor-reactive” T cells in various solid tumor types, including OC. These tumor reactive (TR) TILs exhibit tumor-antigen specificity, MHC dependence, anti-tumor capabilities ex vivo, and suppression of tumor progression in vivo in xenograft models of human OC and melanoma. The capacity of this small number of TR TILs to mediate long-lasting benefit to patients suggests that naturally-occurring tumor-reactive T cells may have inherent capacity for self-renewal, asymmetric division and pluripotent differentiation to terminal effector cell stages. T cells with inherent genetic and proteomic stemness properties, akin to hematopoietic stem cells, have been coined “stem cell memory T cells” (TSCM) and have been described in the circulation of mice and humans. Still, there remains a significant gap in knowledge regarding TSCM cells in human tumors and what role they may play in the control of cancer progression. We hypothesize that tumor-reactive T cells with stem cell properties (TR-SCM) are required for durable anti-tumor responses and improved patient survival. In preliminary results, we compared Tscm T cells in normal and OC patient PBMCs to TILs from OC tumors, using mass cytometry (CyTOF), a multiparameter single cell analysis technique that allows for the simultaneous interrogation of 35+ analytes per cell basis. Our preliminary data demonstrates that (1) Tscm TILs express phenotypic markers expected of Tscm T cells; (2) a subset of Tscm TILs positive for CD45RO exhibit a tumor-reactive phenotype, as they are positive for CD137, IFNg, TNFa, and IL-2; (3) CD45RO positive Tscm TILs express higher levels of PD-1, Lag-3, TIGIT, compared to patient Tscm T cells. Furthermore, a pilot study using CD3/CD28 bead activation supports the idea that Tscm TILs are capable of self-renewal and pluripotent differentiation to terminal effector stages. These results show for the first time that Tscm and TR TILs with features of stemness (TR-SCM) are found within OC patient tumors, and imply that these Tscm TILs are more susceptible to exhaustion than circulating Tscm T cells.

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As development proceeds, inductive cues must be appropriately interpreted by competent tissues for cell specification to occur. While key inductive factors and the signaling pathways within competent cells are fairly well-described, the mechanisms by which tissues lose the ability to respond to inductive signals are not understood. We study the loss of competence to Wnt signaling in dorsal-ventral specification in Xenopus laevis. While localized activation of Wnt signaling during the cleavage stage in X. laevis leads to dorsal development, competence to induce dorsal genes in response to Wnts is lost by the midblastula stage. This loss of competence occurs at the level of Wnt target gene promoters at or downstream of Tcf3, a DNA-binding factor that mediates transcription of Wnt target genes. We hypothesize that loss of competence is mediated by changes in histone modifications at the promoter of Wnt target genes that lead to an increasingly repressive state. In support of this hypothesis, exposure to an HDAC inhibitor extends the window of competence to Wnt pathway activation through the late blastula stage and increases acetylation at the promoter of Wnt target genes.
Natania Field

MVP

The E3 Ubiquitin Ligase Itch limits CD4 T cell proliferation through metabolic independent mechanisms

Natania Field, Joseph Dybas, Emily Moser, Varshini Gali, Omar Elbulok, Paula Oliver

In order to mount a robust anti-pathogen response, effector CD4 T cells must undergo rapid proliferation. However, if T cell expansion is poorly controlled, excess inflammation can occur, damaging the host. One activation-induced change that allows T cells to proliferate rapidly is the upregulation of glycolytic activity. T cells can also regulate their expansion through metabolic-independent processes, such as cell cycle progression. Regulation of both metabolic dependent and independent processes is essential to maintaining T cell homeostasis. One important regulator of CD4 T cell proliferation is the E3 ubiquitin ligase Itch. While the role of Itch in cytokine production has been extensively studied, it is unknown how Itch regulates T cell proliferation, and whether this activity of Itch affects T cell pathogenicity. We have found that Itch deficient T cells proliferate more in vitro and in vivo independently of cytokine production, correlating with increased capacity to cause disease. However, Itch does not directly affect T cell glycolytic capacity nor mitochondrial respiration, and Itch deficient T cells have no changes in mammalian target of rapamycin complex 1 (mTORC1) activity. We plan to analyze the mRNA and protein changes in activated Itch deficient T cells to determine which biological processes are affected by Itch, and which proteins may be targeted for degradation. This project may reveal new mechanisms that limit T cell expansion.
In normal cells mitochondria are the primary energy-producing organelle for most metabolic activities. On the other hand, cancer cells display a remodeled metabolism featured by the production of energy in a manner independent of mitochondria. For many years, it was assumed that the functional role of mitochondria as the “powerhouse of the cell” was diminished in this aberrant state. Nonetheless, in 2016 the Foskett lab discovered that constitutive block of calcium signaling to mitochondria in tumorogenic cells results in a deadly bioenergetic crisis. Therefore, I hypothesize that calcium signaling into mitochondria can be targeted to damage and kill cancer cells. To evaluate the role of calcium signaling into mitochondria in cancer, I will engineer a cancer model in vitro by transforming normal primary mouse fibroblasts into malignant cells through the overexpression of oncogenes. Then, I will genetically knock out (KO) the mitochondrial calcium uniporter (MCU) in transformed cancer-like fibroblasts to eliminate the main calcium influx pathway in mitochondria. Finally, I will determine the effects of MCU KO in the cancer associated properties of transformed fibroblasts in vitro. Overall, this project aims to study the therapeutic potential of targeting calcium signaling into mitochondria through MCU as a new cancer therapeutic.
Ian Folkert

CB

An Interleukin-13-Endothelin 1 Axis in Soft Tissue Sarcoma Pulmonary Metastasis

Ian Folkert, Samir Devalaraja, Jerrick To, Zahidul Alam, Minghong Li, Patricia Young, Mai Dang, Malay Haldar

Soft tissue sarcomas (STS) comprise a heterogeneous group of solid tumors arising from mesodermal tissues, including muscle, adipose, lymphatic and endothelial tissues. Nearly 60% of patients with high-grade tumors will develop metastasis, which is almost uniformly fatal. Lung is the most common site of metastasis, but pathways controlling pulmonary metastasis are poorly understood. Using multiple murine sarcoma cell lines derived from our mouse models of sarcoma, we recently discovered that interleukin 13 (IL-13) significantly increases the expression of the small vasogenic peptide Endothelin 1 (Edn1) in sarcoma cells. IL-13 is a potent cytokine that drives Th2-type immune responses, which are thought to promote tumor progression through a variety of mechanisms. We generated murine sarcoma cell lines expressing shRNAs targeting Edn1, and preliminary data suggest that Edn1 knockdown leads to a decrease in the frequency of lung metastasis. We have also found that Edn1 promotes disruption of microvascular endothelial tight junctions in vitro, suggesting a possible mechanism through which Edn1 promotes sarcoma pulmonary metastasis. Our findings suggest the existence of a previously unknown pathway wherein Th2 cytokines from tumor-infiltrating leukocytes promote lung metastasis by upregulating Edn1 expression in tumor cells.

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Devin Fisher

*MVP*

**Defective viral genome-derived oligonucleotides induce protective antibodies and CD8+ T-cells during vaccination**

Devin G. Fisher, Gaia M. Coppock, Carolina B. López

Many newly developed vaccines use killed pathogens or pathogen subunits as antigens. Unfortunately, these inert antigens do not contain danger signals required to induce robust adaptive immunity. Adjuvants are added to vaccines to provide danger signals that help boost and shape the immune response. Currently, licensed adjuvants can effectively skew the immune response towards type-1 immunity necessary for the clearance of viruses and other intracellular pathogens. How viruses induce type-1 immunity could be leveraged to better understand the mechanisms the induction of adaptive immunity. The primary immunostimulatory molecules during paramyxovirus infections are defective viral genomes (DVGs). We identified the immunostimulatory motif of a DVG from Sendai virus and created a DVG-derived oligonucleotide (DDO) to test as a vaccine adjuvant. Intramuscular injection of DDO with inactivated virus induced robust type-1 immunity characterized by CD8+ T-cell responses and antibodies of the IgG2c isotype. This induction of immunity relies heavily on type I interferon, which is not observed with other adjuvants. Understanding how DDO induces type-1 immunity could lead to more directed vaccine design for specific pathogens.

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Clark Fritsch

*CPM*

**Investigation of chemical-induced readthrough of premature termination codons using single molecule fluorescence resonance energy transfer**

Clark Fritsch, Arpan Bhattacharya, Vijay Singh, Martin Ng, Barry Cooperman, Yale Goldman

Nonsense mutations in DNA result in the replacement of sense codons in mRNA with premature termination codons (PTCs) that prevent the synthesis of full-length proteins during translation. These PTCs are responsible for ~11% of all genetically inherited diseases in humans including cystic fibrosis, Duchenne muscular dystrophy and Hurler syndrome. A common strategy to treat these diseases has therefore been to develop small molecules that suppress nonsense mutations by stimulating the incorporation of non-cognate tRNAs opposite PTCs through a process termed ribosomal readthrough. Although ribosomal readthrough may allow for the synthesis of full-length and active proteins in the presence of PTCs, the mechanisms by which nonsense suppressors stimulate readthrough in eukaryotes is unknown. Here, we demonstrate progress towards the development of a novel in vitro assay that relies on single molecule fluorescence resonance energy transfer measurements to investigate the frequency of translational readthrough of PTCs and the mechanisms by which nonsense suppressors stimulate this readthrough through their interactions with the ribosome. This work will not only contribute to our overall understanding of how ribosomes interact with PTCs, but may also aid in efforts to identify nonsense suppressors that have improved clinical efficacy in humans with diseases caused by nonsense mutations.

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Lipid levels are associated with cardiovascular disease, and previous genome-wide association studies have identified 150+ loci associated with these traits – however, the genetic mechanisms underlying most loci are not well understood. Research indicates that changes in the abundance of alternatively spliced transcripts may be an important mechanism contributing to complex traits. With the increased viability of induced pluripotent stem cells (iPSCs) and iPSC-derived hepatocyte-like cells (HLCs), understanding whether these models can be used to interrogate lipid biology is of increasing interest. Consequently, identifying genetic loci that associate with alternative splicing (i.e., sQTLs) in these cells and determining the degree to which these loci are informative for lipid biology would be ideal, but has not yet been described. We present sQTLs from sample-matched iPSC and HLC lines, and from a separate set of primary liver samples. Genes that are differentially spliced between iPSC and HLC cells are enriched for lipid metabolism pathways. HLC sQTLs co-localize with GWAS lipid loci previously unexplained by HLC eQTLs. HLC sQTLs more closely represent primary liver sQTLs compared to iPSC sQTLs. Our results provide a foundation for efforts that use iPSC and iPSC-derived cells to evaluate genetic mechanisms influencing cardiovascular disease risk.
Emmanuelle Genoyer

MVP

Defective viral genomes alter how Sendai virus interact with cellular trafficking machinery leading to heterogeneity in the production of viral particles among infected cells

Emmanuelle Genoyer, Carolina Lopez

Defective viral genomes (DVGs) generated during RNA virus replication determine infection outcome by triggering innate immunity, diminishing virulence, and, in many cases, facilitating the establishment of persistent infections. Despite the critical role of DVGs during virus-host interactions, the mechanisms regulating production and propagation of DVGs are poorly understood. Visualization of viral genomes using RNA fluorescent in situ hybridization revealed a striking difference in the intracellular localization of DVGs and full-length viral genomes during infections with the paramyxovirus Sendai. In cells enriched in full-length virus, viral genomes clustered in a perinuclear region and associated with cellular trafficking machinery, including microtubules and the GTPase Rab11a. However, in cells enriched in DVGs, defective genomes distributed diffusely throughout the cytoplasm and failed to interact with this cellular machinery. Consequently, cells enriched in full-length genomes produced both DVG and full-length genome-containing viral particles, while DVG-high cells poorly produced viral particles, yet strongly stimulated antiviral immunity. These findings reveal the selective production of both standard and DVG-containing particles by a subpopulation of cells during infection that can be differentiated by the intracellular localization of DVGs, and highlight the importance of considering this functional heterogeneity in analyses of virus-host interactions during infection.

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Alexis Gibson

*MVP*

**Genome Wide CRISPR/Cas9 Knockout Screen Identifies Host Genes Important for Cryptosporidium parvum Infection**

Alexis Gibson, Adam Sateriale, Boris Striepen

Cryptosporidium is an obligate intracellular protozoan parasite, and the second leading cause of severe diarrhea and diarrheal-related death in children worldwide. There are currently no vaccines and the only drug available has low efficacy in immunocompromised individuals. A lack of tools to study Cryptosporidium has impeded progress towards development of novel therapeutics. Due to these challenges, little is known about the host response to Cryptosporidium. We conducted a genome-wide CRISPR/Cas9 knockout screen to discover genes necessary for Cryptosporidium parvum infection. HCT8 cells stably expressing Cas9 were transfected with a lentiviral sgRNA library targeting every gene in the human genome. Selection for resistance to cell death was accomplished by three consecutive 72-hour C. parvum infections. After each challenge, cells were removed for gDNA extraction and sequenced to determine the abundance of each sgRNA. The top 25 enriched genes after three replicates indicate that type III interferon signaling is important for susceptibility to infection. Investigation into the role of type III interferon signaling in C. parvum infection will reveal how a viral defense pathway promotes parasite infection. Genes in pathways of glycosaminoglycan synthesis and glycosylphosphatidylinositol anchor synthesis were also amongst the top candidates, likely responsible for facilitating parasite attachment and invasion.
Stephen Goldstein

MVP

MERS-CoV NS4b accessory protein is a multifunctional innate immune antagonist

Stephen A. Goldstein, Susan R. Weiss

Middle East respiratory syndrome coronavirus (MERS) has caused over 2,000 cases and more than 750 deaths since its 2012 discovery. Like all coronaviruses, MERS encodes lineage-specific nonstructural accessory proteins in the 3’ end of its genome. We previously identified the MERS NS4b accessory protein as an MHV NS2 homolog and described its role as an RNase L antagonist. NS4b and MHV NS2 are 2H-phosphoesterases (PE) that inhibit RNase L by cleaving its activating ligand 2’-5’ oligoadenylate. 2H-PEs are structurally and enzymatically conserved from prokaryotes to mammals, with many involved in RNA processing. Unlike MHV NS2, NS4b contains a nuclear localization signal (NLS), localizes primarily to the nucleus, and cleaves diverse nucleotide substrates, suggesting it performs additional functions. We report that mutation the catalytic site or nuclear localization sequence of NS4b results in elevated levels of interferon mRNA and expression of downstream ISGs independent of RNase L activation. NS4b mutant viruses are modestly attenuated in human airway-derived cells. Intriguingly, RT-qPCR analysis of antiviral transcripts using exon and intron-specific primers suggests NS4b may play a yet uncharacterized role in splicing. This would represent the first known role for viral phosphodiesterase activity outside inhibition of the OAS-RNase L pathway.

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An ERK/hnRNPK/JUND axis regulates pancreatic beta cell survival

Austin L. Good, Corey E. Cannon, Matthew W. Haemmerle, Nicolai M. Doliba, Morris J. Birnbaum, Doris A. Stoffers

In type 2 diabetes, prolonged oxidative stress contributes to the dysfunction and loss of pancreatic beta cells. A highly conserved feature of the cellular response to stress is the regulation of mRNA translation, however, the mechanisms underlying this process in beta cells are not fully understood. Here we use TRAP-seq as a means to discover novel translationally regulated genes in beta cells, leading to the identification of the transcription factor JUND as translationally upregulated in islets during metabolic stress. Depletion of JUND in beta cells reduces oxidative stress and apoptosis caused by high glucose and free fatty acid levels and lowers the expression of several pro-oxidant genes. Further, the RNA binding protein hnRNPK is phosphorylated and post-transcriptionally regulates JUND in a MEK-dependent manner. Importantly, this hnRNPK/JUND axis is activated in islets from diabetic db/db mice and in human islets exposed to metabolic stress. Thus, a translation-centric approach uncovered hnRNPK and JUND as stress-responsive factors in beta cells that contribute to redox imbalance and apoptosis during pathophysiologically relevant stress.
Bone Morphogenetic Protein (BMP) acts as a morphogen to pattern the dorsal-ventral (DV) axis in all vertebrates. In zebrafish, a gradient of BMP signaling activity forms across the embryo during gastrulation. BMP signaling specifies multiple ventral cell fates, whereas suppression of BMP signaling results in dorsal cell fates. However, it is unknown how cells along the DV axis interpret and translate distinct levels of BMP signaling into differential gene activation to specify multiple cell fates. We have identified genes that are directly regulated by BMP signaling by performing RNA-seq on bmp7-/- embryos treated with a translation inhibitor and rescued with BMP2/7 protein injection. We measured the spatial relationship between the BMP transcriptional effector phosphorylated Smad5 (pSmad5) and the boundaries of gene expression and identified multiple genes with expression boundaries that correlate with distinct levels of pSmad5. Specific levels of pSmad5 accurately predict where target genes are expressed in mutants with altered pSmad5 gradients. We have discovered that the pSmad5 gradient contains multiple threshold levels that position the expression of different genes. This work suggests that cells across the DV axis interpret distinct concentrations of nuclear pSmad5 to induce the expression of target genes and position different ventral cell fates.
Fast swimming jellyfish of the phylum cnidaria are the earliest branching metazoan lineage with striated muscle, and they are capable of growing much larger than earlier branching, ciliary-powered metazoan species. The molecular and cellular basis of the evolutionary transition to scalability is poorly understood as these diploblasts all lack true mesoderm and previously recognized sarcomeric scaffolding proteins. Here we show that a broad sampling of cnidarian species express high-molecular weight orthologs to titin, the essential, multi-megadalton protein for sarcomere scaffolding in triploblasts. We find that allometric scaling of sarcomeres in cnidaria is achieved by self-similar (fractal) folding of the epitheliomuscular sheet. Mitochondrial cytochrome c oxidase and ATP synthase are polarized near opposite ends of the anatomical distance between environmental oxygen and the sarcomeric myosin ATPase, thereby facilitating high energy flux in the absence of a circulatory system. The cnidarian proteomes include identifiable orthologs of membrane-associated muscle proteins implicated in a majority of heritable human myopathies including Duchenne muscular dystrophy (DMD), illuminating the ancestral genomic origin of critical sarcomeric proteins. These findings frame the chronology of innovations associated with the emergence of scalable locomotive power in early metazoan evolution, with implications for the mechanobiology of dystrophin and therapy for DMD.
Jessica Grindheim

CB

PRC2 proteins EZH1/2 regulate timely postnatal hepatocyte maturation by repression of euchromatic promoters

Jessica M. Grindheim, Dario Nicetto, Greg Donahue, Kenneth S. Zaret

The inability to derive fully functional cell types, such as hepatocytes, from stem cells may emanate from the lack of knowledge about mechanisms that underlie postnatal cell maturation. We characterized hepatocyte maturation during the postnatal day 14 (P14) to 2-month-old (M2) transition and found more than 3000 genes differentially expressed. Nearly half of such maturation genes have H3K27me3 at their promoters or gene bodies at P14 or M2. Genetic ablation of both PRC2 histone methyltransferases in perinatal livers causes hepatocytes to prematurely differentiate, expressing genes at P14 that would normally be induced later, by M2. Using srHC-seq, a new method to map sonication-resistant heterochromatin, we found that the H3K27me3+ prematurely upregulated genes have euchromatic promoters at P14. We also observed derepression of many non-hepatic lineage genes with euchromatic promoter H3K27me3-marking. Thus, Polycomb repression is used to restrain expression of late liver maturation genes and lineage inappropriate genes at euchromatic promoters.

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Examining the Poly(C) Binding Proteins in Pancreatic Beta cell Homeostasis

Matthew W. Haemmerle, Austin L. Good, Louis R. Ghanem, Stephen A. Liebhaber, Caroline C. Philpott, Doris A. Stoffers

Beta-cells are the only cells in the human body that produce insulin to regulate blood glucose homeostasis. Insufficient insulin output due to beta-cell dysfunction and failure underlies the pathogenesis of Type 2 Diabetes Mellitus. Mounting evidence has suggested that post-transcriptional regulation of gene expression is essential for beta-cell function and survival. However, much of how the post-transcriptional landscape is regulated to promote beta-cell homeostasis remains largely unexplored. Based on translational and RNA-protein studies previously performed in our lab in MIN6 beta-cell insulinoma cells, we have determined that the poly(C) binding protein family of RNA binding proteins, specifically PCBP1 and PCBP2, post-transcriptionally regulate expression of genes implicated in beta-cell stress response and identity. Furthermore, our lab has previously shown in MIN6 cells PCBP1 and PCBP2 depletion impairs glucose stimulated insulin secretion. By using mouse models with beta-cell specific deletion of PCBP1 and PCBP2, we are testing the hypothesis that PCBP1 and PCBP2 post-transcriptionally enhance expression of genes involved in beta-cell stress response and survival to promote beta-cell homeostasis. We expect these studies to advance the physiological and mechanistic understanding of post-transcriptional regulation in beta-cells and to establish a paradigm for PCBP1 and PCBP2 in maintaining beta-cell function and identity.
Christin Herrmann

MVP

Adenovirus E1B55K/E4orf6 enhance viral late protein expression through non-degradative ubiquitination of RNA-binding proteins

Christin Herrmann, Jennifer C. Liddle, Joseph M. Dybas, Benjamin A. Garcia, Matthew D. Weitzman

Viruses such as adenovirus can promote infection by hijacking the host ubiquitination machinery to redirect cellular processes or target anti-viral proteins for degradation. Adenovirus mutants lacking E1B55K or E4orf6, two critical components of an adenoviral ubiquitin ligase, show severe defects in viral late RNA and protein production that are unexplained by currently known substrates. To address this gap in knowledge, we used a proteomics approach to identify host proteins ubiquitinated upon E1B55K/E4orf6 expression and predicted potential degradation by whole cell proteomics. Our analysis revealed an enrichment for RNA-binding proteins, including the closely related proteins RALY and hnRNPC. Neither of these host proteins decrease upon E1B55K/E4orf6 expression or adenovirus infection, making them the first known non-degraded substrates of the adenovirus ubiquitin ligase. Interestingly, while knockdown of RALY and hnRNPC did not impact wild-type virus infection, their depletion resulted in significant rescue of viral late protein expression during infection with an E1B55K-deleted mutant virus that can no longer ubiquitinate substrates. We are currently investigating how non-degradative ubiquitination of RALY and hnRNPC affects RNA processing pathways and contributes to viral late protein production. This research will expand our understanding of how viruses employ the cellular ubiquitination machinery to manipulate the host environment.

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A balance of cytoskeletal forces maintains nuclear architecture in the cardiomyocyte

Julie Heffler, Patrick Robison, Alexey Bogush, Kimberly Veliz, Parisha Shah, Raj Jain, Benjamin L. Prosser

Loss of function mutations in the Linkers of the Nucleo- and Cytoskeleton (LINC) complex that couple the cytoskeleton to the nuclear lamina are associated with dilated cardiomyopathy and disease. While the connections between the LINC complex and the cytoskeleton are well-studied in non-muscle cell types, this topic remains understudied in the heart, where the cytoskeletal structure is by comparison quite unique. Here we sought to determine which components of the cardiac cytoskeleton directly interact with the nucleus and are important for maintaining proper cardiac function and nuclear structure. Acute desmin depletion led to severe lamina infolding and wrinkling of the nuclear membrane. This nuclear infolding in the absence of desmin required an intact MT network, suggesting that desmin normally resists MT-dependent compression of the nucleus. This collapse of the nuclear lamina was associated with large-scale histone remodeling and changes in gene expression measured via RNAseq. Further, these changes in gene expression correspond with decreased excitation-contraction coupling and contractility in desmin depleted cells. Together these data show that the microtubule and desmin intermediate filament networks provide a force balance that is required to maintain basal nuclear architecture and contractile function in the adult heart.
Investigation of in vivo dynamics of self-renewal in murine Lnk-/ Hematopoietic Stem Cells

Nicholas Holdreith, Joanna Balcerek, Jessica Grindheim, Ryan Donaghy, Alexey Bersenev, Wei Tong

Hematopoietic stem cells (HSCs) are rare cells in the bone marrow that are responsible for the continuous generation of all blood cells throughout life and are distinguished by their multilineage differentiation and self-renewal capacities. A central question in HSC biology is how HSCs retain their identity through division. One major factor controlling self-renewal in HSCs is cytokine signaling. Deficiency of the adaptor protein LNK, a negative regulator of the Thrombopoietin (TPO) signaling pathway, results in a 10-fold expanded functional HSC pool with increased propensity for self-renewal. Here we investigate the mechanism by which augmented TPO signaling promotes HSC self-renewal. Using a label retention mouse model that tracks HSC divisional history in vivo, we demonstrate a divisional heterogeneity among phenotypic HSCs. We demonstrate that HSC function is inversely correlated with each in vivo division and that LNK deficiency both enhances the stem cell properties of undivided HSCs, and increases the frequency of bona-fide HSCs in divided cells. We find that division progressively alters cell surface expression of HSC associated markers, and that LNK deficiency promotes retention of these markers. Genome-wide expression profiling reveals progressive reprogramming with division and may represent TPO associated self-renewal target genes.
Endothelial cells require mitochondrial ATP to regulate fatty acid uptake

Ayon Ibrahim, Nora Yucel, Zoltan Arany

Type II diabetes is marked by hyperglycemia brought on by severe insulin resistance, a condition caused in part by intramuscular lipid accumulation. For fatty acids to accumulate in the muscle, they must first be taken up and transported through the endothelial barrier that comprises the capillary wall, a process whose mechanism is not well understood. We conducted a moderate-throughput chemical screen (>2,200 compounds) to identify molecules that modulate endothelial fatty acid uptake. A top hit, niclosamide, a compound known to improve insulin resistance in mice, strongly and rapidly reduces endothelial fatty acid uptake. Niclosamide uncouples mitochondria and lowers membrane potential; furthermore, the capacity of niclosamide and chemical analogues of niclosamide to block fatty acid uptake correlates with their ability to uncouple mitochondria. Other pharmaceutical perturbations of the electron transport chain and oxidative phosphorylation, including inhibition of Complex V and of the adenine nucleotide translocator (ANT), also reduced fatty acid uptake. All of these perturbations reduce production of ATP and its transport to the cytoplasm. The data thus far suggest that ATP generated by mitochondrial oxidative phosphorylation is required to activate endothelial fatty acid uptake.

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A proteomics approach identifies novel proteins in the zebrafish Balbiani body

Allison Jamieson-Lucy, Mary C. Mullins

The Balbiani body is a large, well-conserved, non-membrane bound aggregate found in the zebrafish oocyte. It provides a good model for studying non-membrane bound organelles, but its components remain poorly characterized. Only a handful of resident Balbiani body proteins have previously been identified. We isolated Balbiani bodies from zebrafish oocytes and performed mass spectrometry to define the Balbiani body proteome. Using this technique, we successfully identified over 70 replicable hits representing Balbiani body protein components. Our list includes known Balbiani body proteins and many novel proteins. We have validated Cirbpa, Cirbpβ and Pabpn1l as novel resident Balbiani body proteins by injecting mRNAs encoding fluorescently tagged fusion proteins directly into stage I oocytes and observing Balbiani body localization. This library of Balbiani body proteins is a prerequisite for a comprehensive mechanism for Balbiani body function at a protein level. Next, we will use CRISPR-generated mutants to test the function of these novel proteins within the Balbiani body.
Identifying recurrent mutations from large-scale population sequencing data

Kelsey E. Johnson, Benjamin F. Voight

The presence of recurrent mutations is a strong indicator of disease pathogenicity, and can provide a clue to the underlying causal gene and mechanism contributing to a rare or complex disease. Here, we present a method to infer recurrent mutations in population-level sequencing data without the need for pedigree data or phased haplotypes. The key intuition underlying the method is the observation that the time to the most recent common ancestor (TMRCA) differs between recurrent and identical-by-descent (IBD) mutations. We use a summary statistic for local TMRCA to identify alleles whose measurements are inconsistent with recent IBD as candidate recurrent mutations. We applied our method to SNVs in human whole-genome sequencing data from the UK10K project. We observed a level of enrichment of sequence contexts in putative recurrent mutations that was significantly associated with a sequence context’s substitution probability, including for broader contexts that include three flanking nucleotides. These results suggest we can detect fine-scale differences in mutation rate through our method to identify rare recurrent mutations. Future applications include incorporating recurrent mutation into tests of rare variant burden in disease, and interrogation of the distribution of fitness effects through improved inference of the site frequency spectrum at rare frequencies.
Nadia Kadry

*MVP*

_Elucidating the ability of the HMW₁ and HMW₂ adhesins to protect against nontypeable Haemophilus influenzae colonization of the nasopharynx_

Nadia Kadry, Eric Porsch, Joseph W. St. Geme III

Nontypeable Haemophilus influenzae (NTHi) is a pathogen of significant morbidity in children, causing acute otitis media, sinusitis, conjunctivitis, pneumonia, and in rare cases, invasive disease. NTHi disease pathogenesis begins with nasopharyngeal colonization, followed by spread to sites of disease. There is therefore a need for a vaccine to diminish nasopharyngeal carriage in children and reduce the incidence of associated diseases. NTHi uses several adhesins to mediate nasopharyngeal colonization, including the family of High Molecular Weight (HMW) adhesins, HMW₁ and HMW₂. These adhesins and are the primary targets of convalescent serum antibody in children recovering from H. influenzae infection, suggesting that colonization by H. influenzae is an immunizing event. To explore the protective capacity of the HMW₁ and HMW₂ proteins in vivo, we developed a murine model of immunization and nasopharyngeal colonization by NTHi. Mice immunized with purified protein develop a robust antibody response against HMW₁ and HMW₂; moreover, immunized animals were protected against pulmonary infection by NTHi. These findings highlight the potential for HMW₁ and HMW₂ to stimulate protection against nasopharyngeal colonization.

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Kelsey Kaeding

G&E

Basis for specificity in heterochromatin establishment by zinc finger proteins

Kelsey E. Kaeding, Ryan L. McCarthy, Justin S. Becker, Kenneth S. Zaret

Heterochromatin helps to establish and maintain cell identity by keeping alternate fate genes and repetitive elements tightly repressed. Cell identity can be manipulated through ectopic expression of transcription factors that promote cellular reprogramming, however this process remains both inefficient and imprecise. Our lab has shown that H3K9me3-marked genes that fall within sonication resistant heterochromatin (srHC) pose a barrier to reprogramming by preventing the binding of transcription factors necessary for the reprogramming process. My work aims to understand how specific regions of srHC can be perturbed to allow binding of reprogramming transcription factors, without opening regions that allow for the expression of undesirable genes or repetitive elements. To find locus-specific regulators of heterochromatin, we curated a list of candidates from srHC associated proteins identified by our lab. We performed a functional genetic screen with knockdowns of candidate proteins during human fibroblast to induced hepatocyte reprogramming. This screen revealed that KRAB zinc finger proteins (ZFP) may help to establish or maintain heterochromatin at specific loci. RNA-seq data revealed that different knockdowns allowed different subsets of genes to be activated following reprogramming and showed knockdown specific dysregulation of repetitive elements. Ongoing work aims to determine the specificity of ZFP based heterochromatin establishment.
Exploring the immunometabolism of natural killer T (NKT) cells in neuroblastoma

Neuroblastoma (NB) is a pediatric tumor of the sympathetic nervous system. High-risk NB has a mortality rate of ~50%, underscoring the need for more effective therapies. To explore the potential of NB immunotherapy, we immunophenotyped tumors from an autochthonous murine model of NB (TH-MYCN+/+) to identify the immune cells present in these tumors. Intriguingly, we found a significant frequency of invariant natural killer T (iNKT) cells relative to conventional T cells (Tconv). iNKT cells possess innate-like anti-tumor properties and can engage direct tumor cytotoxicity; importantly, their presence in human NB tumors predicts improved survival. We postulate that the higher frequency of iNKT cells in NB could imply a unique ability to adapt to the harsh, nutrient-poor tumor microenvironment (TME). In Tconv, metabolism is tightly linked to anti-tumor function. However, the metabolic properties of iNKT cells are unknown yet may provide insight into their anti-tumor functions in the TME. Here, we utilize transcriptional approaches to define the metabolic profile of iNKT cells at baseline and under stimulation and understand its link to anti-tumor function compared to Tconv. Collectively, our studies will elucidate novel cellular properties of iNKT cells that could allow for more effective cellular immunotherapy for NB.
Eunsun Kim

CB

β-catenin is recruited to the adherens junction complex and acts as a tumor promoter in HCC

Eunsun Kim, Amanda Lisby, Connie Ma, Nathanael Lo, Ursula Ehmer, Katharina E. Hayer, Emma E. Furth, Patrick Viatour

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths worldwide. Beta-catenin is widely thought to be a major oncogene in HCC based on the frequency of mutations associated with aberrant Wnt signaling in HCC patients. Challenging this model, our data reveal that beta-catenin activating mutations occur early in HCC but only translate into beta-catenin nuclear accumulation during the late stage of the disease. Until then, beta-catenin is primarily located at the plasma membrane in complex with multiple cadherin family members where it drives tumor cell survival by enhancing the signaling of growth factor receptors such as EGFR. Therefore, our study reveals the evolving nature of beta-catenin in HCC to establish it as a compound tumor promoter during the progression of the disease.

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A GATA6 enhancer-associated SNP modulates GATA6 expression during pancreas development and may modulate the penetrance of pancreas agenesis

Siddharth Kishore, Leonardo Cardenas, Paul Gadue

Pancreas agenesis (PA) is a rare genetic disease where the pancreas fails to develop during embryogenesis. The most common cause of PA in humans is heterozygous mutations in GATA6. Interestingly, not all GATA6 heterozygous mutations lead to PA. To further study PA and explain the incomplete penetrance of GATA6 mutations, we generated induced pluripotent stem cell (iPSC) lines from a patient harboring a GATA6 mutation, and used CRISPR-Cas9 genome editing to repair the defect. We also generated embryonic stem cell (ESC) lines carrying an identical mutation. First, we found that the GATA6 mutation on the patient iPSC background presented with a much more severe defect in pancreas development. We also found that the gene corrected patient iPSC line expressed lower levels of GATA6 during pancreas development compared to wild-type ESC lines. To explain this discrepancy, we found a single nucleotide polymorphism (SNP) in a 3' putative enhancer of GATA6, with the iPSC line homozygous for the minor allele variant of this SNP. The lines harboring the minor allele of this SNP expressed GATA6 at lower levels specifically at the pancreas progenitor stage of development. Currently, we are working on identifying transcription factors that could be differentially bound at this region.
Mitochondrial DNA causes changes in nuclear chromatin and makes histone a substrate for histone acetylation


Mitochondrial dysfunction has been recognized as a major driving force in human disease. We have previously shown that a progressive decline in mitochondrial function due to increasing mitochondrial DNA mutation load (heteroplasmy) causes distinct transcriptional changes in the nucleus, however, the mechanism of how mitochondrial dysfunction causes changes in nuclear gene transcription and, as a result, disease remains largely unknown. Here, for the first time, we examine the effect of a human pathogenic mitochondrial DNA mutation at various levels of heteroplasmy on cellular metabolism and nuclear histone modifications. We report that high levels of the mtDNA A3243G tRNALeu(UUR) cause depletion of key tricarboxylic acid cycle intermediates and acetyl-CoA, as well as changes in specific histone acetylation residues. We identify glutamine as a novel substrate for histone acetylation. We hypothesize that the induced global histone changes allow switching on and off whole transcriptional programs, thus efficiently regulating nuclear transcription changes based on the cell’s bioenergetic status.
Marianne Kramer

G&E

Determine the function of the novel long intergenic non-coding RNA At1NC031460 in Arabidopsis

Marianne C. Kramer, Kyle Palos, Eric Lyons, Mark A. Beilstein, Brian D. Gregory

Long intergenic non-coding RNAs (lincRNAs) are an emerging class of molecules that are gaining attention for their roles in many biological processes. There are over 6,000 detectable lincRNAs in Arabidopsis with tissue specific and stress responsive expression patterns. Despite these observations, the functions of most lincRNAs remain unknown. Previously we identified a protein-bound nuclear lincRNA At1NC031460 that is highly conserved in a closest related crop species. To determine the function of this lincRNA, we examined the phenotype of homozygous mutants and found that mutants lacking lincRNA expression were noticeably smaller and developmentally delayed compared to wild-type plants. Interestingly, at1nc031460 mutant plants also begin to senesce earlier than wild-type plants. To examine the molecular function of this lincRNA, we have performed chromatin isolation by RNA precipitation (ChIRP) followed by DNA sequencing. With this technique, the lincRNA is pulled down using biotinylated probes complimentary to the lincRNA and the associated DNA is identified by high-throughput sequencing (ChIRP-seq). From ChIRP-seq, we found At1NC031460 binds to 94 genes which are enriched for proteins that are involved in phospholipid biosynthesis and cuticle development. Given the phenotype of early senescence it appears that At1NC031460 might function in cell wall maintenance and senescence.
Terra Kuhn

*CPM*

**Chromatin-bound nuclear pore proteins recruit the PBAP chromatin-remodeling complex to induce DNA decondensation**

Terra Kuhn, Alejandro Gozalo, Pau Pascual-Garcia, Shawn Little, Maya Capelson

Nuclear pore complexes (NPCs) canonically regulate nucleocytoplasmic traffic, but in recent years, it has become clear that NPCs and their constituent Nups interact with the genome. Depletion of Nups can result in loss of open chromatin state, decreased RNA polymerase II recruitment, and lower target gene expression. How Nups regulate these processes however is not known. Using transgenic Drosophila and a lacI-LacO tethering system, we have observed that nucleoporin Sec13 is sufficient to induce visible chromatin decondensation using larval salivary gland polytene chromosome immunofluorescence. Interestingly, this phenomenon appears independent of recruitment of transcriptional machinery or nuclear positioning. Furthermore, Sec13 recruits proteins associated with formation of open chromatin, including GAGA Factor and components of the PBAP chromatin-remodeling complex, to this locus. Interestingly nucleoporin Elys is also recruited to this locus. In Drosophila cell culture, endogenous Elys, Sec13 and PBAP components interact, with Elys interaction being stronger. Additionally, loss of Elys in Drosophila cell culture results in a genome-wide increase in chromatin compaction as assayed by MNase digestion. Together these experiments elucidate an intranuclear role for nucleoporins in recruiting specific chromatin remodelers to facilitate chromatin decondensation, regulating a crucial step of an early stage in chromatin state and gene activation.

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Activity-dependent regulation of autophagosome dynamics in neurons

Vineet Vinay Kulkarni, Sandra Maday

Neurons fire action potentials at very high frequencies (up to 100 impulses/sec!), which can lead to overuse and damage of synaptic proteins and organelles. Robust quality control pathways are therefore essential to maintain the integrity of the synaptic proteome in response to neuronal activity. Neurons are especially reliant on autophagy, a lysosomal degradation pathway that recycles damaged proteins and organelles. Indeed, neuron-specific knockout of autophagy-related genes leads to neuronal dysfunction and death. However, little is known about how autophagy is regulated in response to changes in neuronal activity. Here, we use live-cell microscopy to define the nature and dynamics of autophagic vesicles (AVs) in neurons under various modalities of neuronal activity. We find that under basal spontaneous activity, AVs in dendrites are predominantly mature autolysosomes that exhibit bidirectional movement. Strikingly, increasing neuronal activity dampens AV motility specifically within dendrites but not in axons. Preliminary data indicate that this activity-dependent decrease in dendritic AV motility can be restored by silencing neuronal activity. Together, we propose a model where neuronal activity can control AV transport specifically in post-synaptic compartments and induce recruitment of AVs to synapses for local regulation of the synaptic proteome.
Expanding AAV tropisms using a novel screening method

Jon Lang, Marie Atilus, Beverly Davidson

Conventional methods to describe adeno-associated virus (AAV) vector tropisms are based on the high, stable expression of a reporter gene in the AAV transgene cassette. As a consequence, conventionally described AAV tropisms omit cell types that exhibit transient or low transgene expression. This creates a potential blind spot when AAV vectors deliver genome editing machinery, because in these cases, only minimal transgene expression is required for editing. To address this problem, we developed a new method to expand known AAV vector tropism that captures both stable, high transgene expression (conventional tropism) as well as transient or low, stable expression (expanded tropism). We demonstrate the superiority of our method to traditional screening methods in a side-by-side comparison. We demonstrate improved detection of AAV8 transgene expression in tissues of known tropism, and we reveal previously unknown cell-type specific AAV8 tropisms. We anticipate that this system will provide new avenues for AAV vector development and improve the application of these vectors for genome editing applications.
Investigating the role of VPS33A in melanosome biogenesis and function

Linh T. Le, Megan K. Dennis, Shanna L. Bowman, Richard A. Spritz, Michael S. Marks

Specificity in membrane fusion is essential for proper organelle maturation. The homotypic fusion and vacuolar protein sorting (HOPS) complex facilitates the tethering of apposing membranes, promotes SNARE complex assembly, and is required for membrane docking and fusion in the classical endo-lysosomal system; however, it is not known how HOPS functions in specialized cell types to facilitate the biogenesis of cell type-specific lysosome-related organelles. Buff (bf) mice, which have a missense mutation in the HOPS complex subunit Vps33a, are hypopigmented, suggesting that the HOPS complex plays a specific role in the biogenesis of melanosomes, which are lysosome-related organelles required for melanin synthesis and storage. To understand the function of Vps33a in the melanosomal pathway, we analyzed immortalized melanocytes from bf mice (melan-bf cells). Unexpectedly, we show by light and electron microscopy that these cells are hyperpigmented with large, mature melanosomes localized to the cell periphery and there is a lack of early stage melanosomes in these cells. Using immunofluorescence microscopy, we show that lysosomal proteins colocalize with pigment granules in melan-bf cells, indicating defective trafficking to melanosomes. Our studies suggest that the HOPS complex has a specific and unexpected role in regulating the maturation of melanosomes.
Intertumoral heterogeneity - the biological and functional differences between individual tumors - poses a challenge for immunotherapy, where only a fraction of patients responds. To understand the factors underlying the heterogeneity of tumor immunity and sensitivity to immunotherapy, we established a library of congenic pancreatic tumor cell clones that recapitulate T-cell-inflamed and non-T-cell-inflamed tumor microenvironments, with distinct patterns of infiltration by T cells, dendritic cells, and myeloid cells. We found that the non-T-cell-inflamed phenotype is dominant over the T-cell-inflamed phenotype and that both quantitative and qualitative features of intratumoral CD8+ T cells determine response to therapy. An integrated transcriptomic/epigenetic approach revealed tumor-cell-intrinsic production of CXCL1 as a major determinant of the non-T-cell-inflamed microenvironment, and ablation of CXCL1 promoted T cell infiltration and sensitivity to immunotherapy. These results demonstrate that heterogeneity of tumor immune phenotypes is driven by tumor cell-intrinsic factors that can be manipulated to influence the outcome of immunotherapy.
DNA double-strand breaks are typically repaired by nonhomologous end-joining or homologous recombination. An intricate cascade of signaling events on the chromatin regulates the balance between these two repair pathways, and disruption of the proper chromatin milieu can perturb this balance. To identify the histone modifications and associated chromatin remodeling events that contribute to the recombination vs. end-joining repair pathway choice, we developed a protocol to isolate mono-nucleosomes associated with pro-recombination and pro-end-joining proteins coupled with histone mass spectrometry to assess the relative abundance of different modifications on these associated nucleosomes. Our method corroborates the known histone marks that correlate with recruitment of the pro-end joining protein 53BP1, and presents a novel approach to studying the chromatin determinants for recruitment of different DNA repair proteins.
Calcineurin plays a key role in maintaining fibroblast homeostasis

Fibroblast activation is a crucial step in tumor growth and metastatic progression. Activated fibroblasts are known to remodel and process extracellular matrix (ECM) and have been shown to function in both pro and anti-tumor roles. However, the cellular and molecular mechanisms that maintain the homeostatic, un-activated state of fibroblasts are not well defined. The Ser/Thr phosphatase calcineurin (CN) and its downstream target nuclear factor of activated T cells (NFAT) play key roles in endothelial cell and immune cell activation, but the role of the CN axis in stromal cells is not known. Here we demonstrate that deletion of CN in vitro alters fibroblast morphology and function consistent with an activated phenotype. CN-null fibroblasts have greater migratory capacity, increased collagen secretion and remodeling, and promotes robust angiogenesis in vitro. ECM derived from CN-null fibroblasts have increased collagen content and greater alignment, altering the morphology and cytoskeletal architecture of both fibroblasts and tumor cells cultured on these matrices. In addition, mice with stromal CN deletion have a greater incidence of and larger lung metastases. Our data suggest that deletion of CN leads to a state of activation in the absence of exogenous activating stimuli.
Lillian Lim

**DSRB**

**Mechanisms of lymphangiogenesis in wound healing**

Lillian Lim, John Welsh, Hung Bui, Jisheng Yang, Li Li, Min Min Lu, Mark Kahn

During development, VEGFC is crucial for lymphangiogenesis, and mice lacking Vegfc have a complete absence of lymphatic vessels. VEGFD, another member of the VEGF family, can also stimulate lymphatic vessel growth; however, the loss of VEGFD has no effect on lymphangiogenesis during development. VEGFC and –D are synthesized as propeptides, and required processing by proteases in order to bind and signal to VEGFR3, expressed primarily on lymphatic endothelial cells (LECs), to drive lymphangiogenesis. VEGFC is cleaved by ADAMTS3, while VEGFD is cleaved by thrombin and plasmin. Thrombin and plasmin are serine proteases present in high concentration at wound sites, suggesting that VEGFD may be the key driver of lymphangiogenesis during wound healing. However, platelets, which are also present in blood clots at wound sites, express VEGFC. Thus, our hypothesis is that both VEGFC and VEGFD may be important in lymphangiogenesis during wound healing, and we address this question using two different wound healing models. Preliminary data suggests that VEGFD is not required in both wound models, while VEGFC is required.
Mei Lin

CB

Investigating the role of TNFAIP8 in phospholipid signaling and carcinogenesis

Mei Lin, Youhai Chen

Tumor necrosis factor alpha-induced protein 8 (TNFAIP8)-like family is a newly described group of proteins implicated in tumorigenesis and inflammation. TNFAIP8 is frequently over-expressed and a potential risk factor for the progression and poor prognosis of several malignancies. Phosphatidylinositol (PtdIns) is a membrane phospholipid that can be phosphorylated at the 3, 4 and 5 positions of the inositol ring to generate seven phosphoinositides, among which PtdIns(4,5)P2 is the most abundant and binds to proteins important for actin polymerization, focal contacts formation and cell adhesion. Phosphoinositide 3-kinase (PI3K) generation of PtdIns(3,4,5)P3 and the subsequent activation of downstream cascades compose the signaling axis controlling cell survival and growth. Recent research reveals that the crystal structure of TIPE homology domain contains a large hydrophobic central cavity which may confer lipid binding capacity. We find TNFAIP8 can recognize and bind to PtdIns(4,5)P2 and PtdIns(3,4,5)P3 present in the model lipid layers. Furthermore, we find TNFAIP8 promotes the phosphorylation of PI(4,5)P2 by PI3K (p110a/p85a) in a dose-dependent manner in vitro. TNFAIP8-deficient HL-60 cells exhibit decreased growth and upregulated cofillin and PAK signaling. These studies constitute a step forward in characterizing the precise function of TNFAIP8 in carcinogenesis and its mechanisms of actions.

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Ricardo Linares

*G&E*

**Defining the role of HOPX and genome-nuclear lamina interactions in cardiomyocyte commitment**

Ricardo Linares, Parisha Shah, Qiaohong Wang, Jon A. Epstein, Rajan Jain

Progressive lineage restriction occurs as undifferentiated cells develop into mature cell types. During cardiogenesis, multipotent cardiac progenitors (CPCs; marked by Isl1+) give rise to endothelial, smooth muscle, and cardiomyocyte lineages. It has been shown that Hopx (Homeodomain-Only Protein X) expression defines a pool of CPCs that exclusively gives rise to cardiomyocytes. However, it is not well understood if and how Hopx functions to restrict cell fate choice during cardiogenesis. Hopx deletion during cardiac differentiation in mouse embryonic stem cells results in aberrant expression of alternative lineage markers, such as canonical endothelial genes. In addition, preliminary experiments indicate Hopx interacts with nuclear lamina proteins, leading us to hypothesize that Hopx restricts alternative lineage choices through repression of gene regulation at the nuclear lamina. Studies to date suggest that a subset Hopx-/- cells cannot faithfully commit to the myocyte lineage and instead adopt an endothelial cell fate in vivo. Ongoing experiments are aimed at quantifying lineage adoption in vitro and mapping changes regions of the genome that associate with the nuclear lamina upon loss of Hopx during cardiogenesis. Taken together, our results will advance our understanding of how nuclear architecture shapes fate adoption during cellular commitment and differentiation.

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Chromatin architecture and transcription are intimately linked, with CTCF being one of the most extensively characterized mediating factors. Although CTCF engages in a wide range of activities, ranging from higher order chromatin domain insulation, long-range looping interactions, transcription pausing/pause release, and transcription activation/repression, the context and mechanisms of each function remain poorly understood. Here, we aim to disentangle CTCF functions in chromatin architecture and transcription through a loss-of-function approach. Using Auxin-inducible degron (AID) system in a well-established mouse erythroid cell line, JC4, we observed rapid and global CTCF depletion. Surprisingly, we observe a subpopulation of CTCF sites resistant to Auxin-mediated CTCF degradation. These sites exhibit unique features in CTCF binding motif, genome distribution, histone signature, enrichment with structural proteins, and proximity to domain boundaries. It is tantalizing to hypothesize that these stable CTCF sites perform distinct transcriptional/architectural functions. Additionally, we observe subtle yet global transcriptional changes associated with CTCF degradation. Both RNA polymerase II recruitment at the promoter and pausing/pause release contribute to significant transcriptional changes of a subset of genes. Ongoing work aim to identify architectural changes (or lack thereof) underlying transcriptional changes.
Quentin McAfee

*CPM*

**Detecting toxic titin fragments in peripartum cardiomyopathy**

Quentin McAfee

Truncating variations in titin (TTNtv) are associated with peripartum cardiomyopathy (PPCM), but it is unknown whether TTNtv cause PPCM via gain of function or haploinsufficient mechanisms. TTNtv are present in skeletal muscle from TTNtv humans, and ribosome sequencing demonstrates TTNtv translation in rat hearts, but immunoblotting does not detect the expected size peptide in rat or human hearts. I therefore hypothesize that cardiac TTNtv are translated but TTNtv sub-fragments cause PPCM. Because fragmentation of titin may prevent immunoblot detection of TTNtv and standard sequencing approaches can't differentiate normal from truncated titin, I am pursuing three approaches to detect TTNtv or fragments thereof in TTNtv PPCM models: First, proteomic and antibody detection of a unique peptide sequence induced at a TTNtv truncation site found in a PPCM patient. Second, crossing of highly polymorphic CAST/EiJ mice with C57BL/6-WT/TTNtv mice to permit allelic differentiation TTN from TTNtv by sequencing. Third, GFP and epitope tagging of TTNtv will allow direct TTNtv detection. These methods should allow detection of truncated titin fragments against a background of wild type titin and provide a methodological foundation upon which to address the question of whether truncated titin fragments may be the mechanistic cause of peripartum cardiomyopathy.
Autophagy protects against alcohol-induced acute mitochondrial damage and metabolic stress in esophageal keratinocytes

Prasanna M. Chandramouleswaran, Noah Engel, Koji Tanaka, Satish Srinivasan, Manti Guha, Clementina A. Mesaros, Hiroshi Nakagawa

Alcohol consumption is a major risk factor for esophageal squamous cell carcinoma (ESCC). However, the mechanisms underlying alcohol-induced cellular injury, tumorigenesis and compensatory responses are poorly understood. Mitochondria are a major site of alcohol metabolism and are susceptible to alcohol-induced damage. Autophagy is a cyto-protective mechanism that removes damaged cellular components and reduces reactive oxygen species (ROS). We investigated how esophageal keratinocytes respond to alcohol induced oxidative stress and mitochondrial damage. Flow cytometry analysis identified alcohol-induced mitochondrial membrane depolarization coupled with mitochondrial superoxide generation in C57BL/6J murine esophageal keratinocytes in vivo, and in human esophageal keratinocytes in vitro. Alcohol induced functional mitochondrial damage was corroborated by decreased cellular respiration measured via real-time respirometry, and metabolomics. Pharmacological and genetic inhibition of macroautophagy and mitophagy enhanced alcohol-induced depolarized cell population and promoted cell death. Alcohol-induced autophagy was associated with activation of AMPK and decrease in mTORC1 signaling. Furthermore, Alcohol promoted transcriptional activation of autophagy and lysosomal genes associated with TFEB pathway activation. In summary, Autophagy is activated in the context of alcohol-induced mitochondrial stress possibly via the AMPK/mTORC1/TFEB axis to alleviate metabolic and oxidative stress in esophageal keratinocytes.
Ernest Monahan-Vargas

*DSRB*

**Targeting the RNA Repair Pathway to Enhance Axonal Regeneration: Downstream Effectors**

Ernest Monahan-Vargas, Michael Cory, John Karanicolas, Yuanquan Song

RNA 3′terminal phosphate cyclase has been shown to be a conserved regulator of axonal regeneration. Using a virtual screening approach, we have identified potential small-molecule inhibitors of human RtcA to be tested in mammalian neuronal microfluidic chambers. Due to the lack of understanding of the functional importance of these RNA modifications in injury and disease and with the goal of identifying factors essential for axon regeneration, we have utilized a Drosophila sensory neuron injury model—that parallels mammalian injury phenotypically and molecularly—in a candidate-based screen, and identified potential downstream targets. Loss of the microtubule-binding protein tppp led to an inability of peripheral sensory neurons to regenerate while ectopic expression in CNS injury paradigms, led to enhanced regeneration. TPPP localizes to the distal axon after injury, suggesting a role in axonal extension. Finally, we observed an increase in regeneration in HDAC6KO lines—previously shown to be an autophagy regulator and inhibited by TPPP. By taking advantage of the power of fly genetics to identify novel factors and mammalian injury models, this combinatorial strategy offers a unique opportunity to characterize conserved mechanisms of neuronal regeneration. Our results also highlight the potential to target components of quality control mechanisms to promote axonal regeneration.

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Duchenne Muscular Dystrophy (DMD) is a sex-linked disorder characterized by progressive muscle weakness and wasting, ultimately leading to premature death due to respiratory and cardiac complications. DMD is caused by mutations in the DMD gene that results in the absence of the critical 427 kd cytoskeleton protein product, dystrophin. Therapeutic approaches introducing recombinant dystrophin can be subject to immune recognition and destruction. Therefore, our lab has focused on an alternative approach that utilizes dystrophin's paralog protein, Utrophin. Here we show that a synthetic version of utrophin encoded by an Adeno Associated Virus 9 (AAV9-μUtrophin) in which the rod domain has been internally deleted is a highly functional, non-immunogenic substitute for dystrophin, preventing the most deleterious histological and physiological aspects of muscular dystrophy in small and large dystrophic animal models. In a stringent test of immunogenicity, focal expression of μUtrophin in the deletional-null German Shorthaired Pointer dog model produced no evidence of cell-mediated immunity, in sharp contrast to the robust T cell response against similarly constructed μDystrophin. These findings support a model in which utrophin-derived therapies can be used to treat clinical dystrophin deficiency, with a favorable immunologic profile and preserved function in the face of extreme miniaturization.
Somdutta Mukherjee

DSRB

Identifying the relationship between TBX3 and PDX1 during endoderm patterning

Somdutta Mukherjee, Sidharth Kishore, Deborah French, Paul Gadue

Although the signaling molecules and pathways that are involved in endoderm patterning have been studied extensively, the factors that define the pancreatic versus hepatic domains remain elusive. Using human pluripotent stem cells (hPSCs) we aim to further define how cells are specified to either a pancreatic or a hepatic fate. PDX1 is a known regulator of pancreatic development, while TBX3 is involved in regulating liver development. We used CRISPR/Cas9 genome editing technology to generate a TBX3-/hPSC line. We differentiated this line down a hepatic lineage, and found that TBX3-/cells differentiate less efficiently to hepatocytes than wild type cells. Additionally, during the differentiation the TBX3-/cells express PDX1, which is not expressed in hepatocytes. This suggests that TBX3 may be regulating liver development by suppressing PDX1 expression, and therefore suppressing a pancreatic fate.

Additionally, it has been shown that PDX1 represses liver specific gene expression, thus suppressing a hepatic fate. These studies aim to elucidate whether PDX1 and TBX3 regulate each other to specify pancreatic and hepatic domains respectively. This will enhance our understanding of endoderm patterning during development, thus improving in vitro derived cell types that can be used in stem cell based therapies in the future.

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Vanessa Munoz  

*MVP*  

**Surface polysaccharides promote innate immune evasion by pediatric pathogen *Kingella kingae***  

Vanessa L. Munoz, Eric A. Porsch, Joseph W. St. Geme III  

*Kingella kingae* is a gram-negative coccobacillus that is being recognized increasingly as an important cause of invasive disease in young children. The pathogenesis of *K. kingae* disease involves colonization of the posterior pharynx, invasion into the bloodstream, and dissemination to distant sites of infection. *K. kingae* produces a polysaccharide capsule and exopolysaccharide that are required for virulence in an infant rat infection model. In this study, we sought to determine the mechanisms of *K. kingae* resistance to neutrophil-mediated. Experiments with neutrophils demonstrated the critical role of capsule in neutrophil evasion. This finding was further emphasized by the enhanced neutrophil oxidative burst response in the presence of capsule-deficient strains and serum opsonins. To elucidate the mechanism of neutrophil-mediated killing, we inhibited phagocytosis, which resulted in enhanced survival of the capsule-deficient strains. Lastly, to determine the role of surface polysaccharides in antimicrobial peptide resistance, we performed bactericidal assays and uncovered a significant role for exopolysaccharide in survival in the presence of antimicrobial peptides. Based on these results, we conclude that capsule and exopolysaccharides are important virulence factors in vivo and play a critical role in promoting *K. kingae* survival in the presence of neutrophils and antimicrobial peptides.
Danielle Murashige

Promotion of cardiac branched chain amino acid metabolism does not prevent heart failure

Danielle Murashige, Cholsoon Jang, Michael Neinast, Qingwei Chu, Atsushi Hoshino, Tao Wang, Josh Rabinowitz, Zoltan Arany

Elevations in plasma branched chain amino acids (BCAAs) are associated with, and may precede, heart failure (HF). In mice, inhibiting whole-body BCAA catabolism worsens failure, whereas promoting systemic BCAA breakdown preserves cardiac performance during challenge. It is unknown, however, whether alterations to cardiac BCAA metabolism contributes to HF. The first objective of this project is to determine whether promoting cardiac BCAA catabolism alone is sufficient to preserve cardiac function during challenge. The protein kinase BCKDK inhibits the rate-determining step of BCAA oxidation. To promote cardiac BCAA catabolism, we made a cardiac BCKDK -/- mouse and subjected WT and KO mice to isoproterenol. WT and cardiac BCKDK -/- mice showed similar induction of HF markers and similar degrees of ventricular hypertrophy and dilation; however left ventricular ejection fraction and fractional shortening were unaffected. This preliminary study demonstrated that promotion of cardiac BCAA catabolism did not prevent cardiac remodeling; however, isoproterenol challenge was not sufficient to affect contractility in either group. Follow-up studies include a comparison of the response of WT and cardiac BCKDK -/- animals to combination transverse aortic constriction and in vivo metabolic tracing of BCAAs at baseline and during the progression of heart failure.

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Elevations in branched-chain amino acids (BCAAs) are associated with numerous systemic diseases, including cancer, diabetes, and heart failure. However, an integrated understanding of whole-body BCAA metabolism remains lacking. Here, I employ in vivo isotopic tracing to quantify BCAA oxidation in numerous organs in healthy and insulin-resistant mice. I find that most tissues rapidly oxidize BCAAs into the TCA cycle, accounting for 1-6% of TCA carbons, but the pancreas generates a striking 20% of its TCA carbons from BCAAs. Accounting for metabolic rate and total mass of each organ, the greatest quantity of BCAA oxidation occurs in muscle, brown fat, liver, kidneys and heart. In addition, measurements of protein synthesis in the same mice demonstrate the flux of BCAA oxidation is not correlated to protein synthesis. Genetic and pharmacologic suppression of BCKDK, a clinically targeted regulatory kinase, induces BCAA oxidation primarily in skeletal muscle of healthy mice. BCAA oxidation increases in cardiac and skeletal muscle of healthy mice in response to insulin, whereas chronically insulin-resistant mice have blunted BCAA oxidation in adipose tissues and liver, shifting BCAA oxidation toward muscle. Together, this work provides a quantitative framework for understanding systemic BCAA oxidation in health and insulin resistance.
Son Nguyen

*MVP*

**Defining the functional and transcriptional identities of CD8+ T-cell response in lymph nodes of HIV elite controllers**

Son Nguyen, Claire Deleage, Samuel Darko, Amy Ransier, Duc Phan Minh Truong, Perla Del Rio-Estrada, Ali Naji, Gustavo-Reyes Terán, Jacob D. Estes, Daniel C. Douek, Steven G. Deeks, Marcus Buggert, Michael R. Betts

Studies in the peripheral blood have strongly correlated viral control in HIV elite controllers (EC) to effector functions of CD8+ T cells. However, the vast majority of HIV replication in EC likely occurs in lymphoid tissues, where CD8+ T cell control mechanisms remain undefined. Here we directly assess functional and phenotypic properties HIV-specific CD8+ T cells in lymph nodes (LN) of EC. We demonstrate that cytolytic CD8+ T cell cells are almost absent in LN of EC, compared to higher frequencies in viremic HIV chronic progressors (CP). Instead, EC exhibit higher magnitudes of HIV-specific CD8+ T cells and increased non-cytolytic polyfunctional responses compared to CP and ART-treated individuals (ART) in LN. Moreover, LN HIV-specific CD8+ T cells from EC have a distinct transcriptional signature compared to CP defined by increased gene expression of specific cytokines and ribosomal protein subunits and lower expression of inhibitory receptors and pro-apoptotic genes. Together these findings redefine previous concepts of HIV immunopathogenesis, and suggest that HIV-specific CD8+ T cell responses directly responsible for controlling viral replication in lymphoid tissues include unique non-cytolytic functional features and a high capacity to translate mRNA into protein upon antigen encounter.

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Ca2+ uptake into mitochondria occurs through the Mitochondrial Calcium Uniporter (MCU), promoting aerobic respiration and energy production. Nevertheless, excessive matrix [Ca2+] ([Ca2+]m) is toxic, triggering mitochondrial Permeability Transition Pore (mPTP) opening and depolarization of the inner membrane potential. Thus, specific MCU inhibitors are of interest as therapeutics for ischemia-reperfusion injury, neurodegeneration, and cancer. DS16570511 (DS) is a recently identified MCU inhibitor with an IC50 for channel blockade of 7 micromolar. Using permeabilized cell assays to simultaneously monitor mitochondrial Ca2+ uptake and membrane potential, we show that whereas DS inhibits MCU, it is not specific. DS promotes excessive mitochondrial depolarization in response to elevated cytoplasmic [Ca2+] ([Ca2+]c), leading to overestimates of its inhibitory activity on MCU. At low [Ca2+]c when MCU is inactive, DS hyperpolarizes mitochondria and increases the driving force for Ca2+ uptake. MCU activation in the presence of DS-induced hyperpolarization increases [Ca2+]m, triggering mPTP opening. With mPTP inhibited by Cyclosporin A, the IC50 of DS for MCU inhibition is reduced to ~30 micromolar. Whole-mitoplast patch clamp, which experimentally controls membrane potential, reveals reversible inhibition of MCU by 10 micromolar DS. These findings highlight the importance of screening potential MCU inhibitors for off-target effects, particularly on mitochondrial membrane potential.
Transcriptome analyses of somatotropes and lactotropes reveal novel regulators of cell identity in the pituitary

Michael Peel, Yugong Ho, Stephen Liebhaber

The differentiation of the hormone-producing cell lineages of the anterior pituitary represents an informative model of mammalian cell fate determination. The generation and maintenance of two of these lineages, the growth hormone (GH) producing somatotropes and prolactin (PRL) producing lactotropes, are dependent on the pituitary-specific transcription factor, POU1F1. While POU1F1 is expressed in both cell types, and plays a direct role in the activation of both the Gh and Prl genes, GH expression is restricted to somatotropes and PRL expression is restricted to lactotropes. These observations imply the existence of additional, cell type-enriched factors, that contribute to the somatotrope and lactotrope cell identities. Here, we use transgenic mouse models to facilitate sorting of somatotrope and lactotrope populations based on the expression of fluorescent markers expressed under Gh and Prl gene transcriptional controls. The limited number of divergent mRNAs between the two populations includes a set of transcription factors that may have roles in pituitary lineage divergence, and/or in regulating expression of cell-type specific genes after differentiation. Here we highlight one of the lactotrope-enriched factors, Nr4a2, and its impact on the lactotrope lineage as a positive regulator of the prolactin gene.
Ian Penkala

DSRB

Investigating the alveolar response to neonatal hyperoxic lung injury

Ian J. Penkala, Aravind Sivakumar, Josh Pankin, Katharine Stolz, David Frank, Edward E. Morrisey

Formation of the architecturally complex gas exchange surface of the lung requires precise temporal and spatial control of alveolar type 1 (AT1) and type 2 (AT2) cell differentiation. Hyperoxia exposure is a common neonatal lung injury that disrupts normal developmental processes. A better understanding of repair processes after neonatal lung injury could yield key insight into novel therapies to regenerate and mature the neonatal lung. We subjected a variety of genetic mouse models to hyperoxia to determine neonatal alveolar epithelial cell responses to hyperoxic injury and investigate signaling pathways essential for repair. We aimed to assess effects of hyperoxia on alveolar cell fate, proliferation, and alveolar repair. Neonatal hyperoxic lung injury results in significantly enhanced epithelial plasticity. As previously reported, the number of AT2 cells increased after neonatal hyperoxic lung injury and returned to levels comparable to those in the uninjured mice at the completion of alveolar maturation. Interestingly, we report a persistent decrease in the number of AT1 cells present after injury compared to uninjured mice. We demonstrate that neonatal hyperoxic lung injury can irrevocably affect the balance of AT1 and AT2 cells present in the postnatal lung that may impair lung function or repair in adult animals.
Alexandra Perry

MVP

The Kingella kingae PilC1 MIDAS motif is essential for type IV pilus adhesive activity

Alexandra Perry, Eric A. Porsch, Joseph W. St. Geme III

Kingella kingae is a gram-negative bacterium that colonizes the upper respiratory tract in children. Although usually present as a commensal organism, it can breach the epithelial barrier and migrate to other areas of the body, causing diseases. Type IV pilus (T4P)-mediated adherence to host respiratory epithelium is the first step in colonization, a prerequisite for invasive disease. The pilus-associated proteins PilC1 and PilC2 promote adherence, with at least one of these proteins being required for adherence to host cells in vitro. The mechanism by which PilC proteins promote adherence remains unknown. PilC1 is predicted to contain a metal ion-dependent adhesion site (MIDAS) motif. We hypothesized that the PilC1 MIDAS motif is directly involved in mediating T4P adhesive interactions. A PilC1 MIDAS mutant was unable to adhere to human epithelial cells or mediate twitching motility in vitro, although no defect in piliation or pilus retraction was observed. Additionally, while PilC1 was capable of mediating adherence to extracellular matrix components in vitro the MIDAS mutant was not. These data suggest a dual role for the K. kingae PilC1 MIDAS motif in adherence and twitching motility, supporting our hypothesis that the PilC1 MIDAS motif is directly involved in mediating adhesive interactions.
Benjamin Philipson

CPM

4-1BB-CAR-Mediated Non-Canonical NF-kappaB Signaling Enhances CAR T cell Survival and Suppresses Bim Expression

Ben Philipson, Roddy O’Connor, Michael J. May, Carl H. June, Steven M. Albelda, Michael C. Milone

Chimeric Antigen Receptor (CAR) T cell therapy induces deep and durable responses in a large percentage of patients with B-cell malignancies. These responses often correlate with CAR T cell persistence in patients. 4-1BB CAR (BBzeta) T cells persist longer than CD28 CAR (28zeta) T cells in patients. Endogenous 4-1BB, but not CD28, activates the non-canonical NF-kappaB (ncNF-kappaB) pathway, which is associated with cell survival. Therefore, we hypothesize that the BBzeta, but not the 28zeta CAR, activates ncNF-kappaB signaling, which promotes CAR T cell persistence by suppressing pro-apoptotic gene expression. Using primary human T cells, we generated 28zeta or BBzeta T cells by lentiviral transduction. Following CAR activation, ncNF-kappaB signaling was detected in BBzeta but not 28zeta T cell lysates. Control BBzeta T cells expanded approximately 10 fold more than BBzeta T cells in which ncNF-kappaB signaling was blunted. These cells exhibited higher rates of cell death and expressed substantially more of the pro-apoptotic protein, Bim, relative to control. Therefore, BBzeta, but not 28zeta signaling activates the ncNF-kappaB pathway, which protects BBzeta T cells from cell death likely by restricting the expression of the pro-apoptotic protein, Bim.
Intracellular bacterial pathogens are responsible for significant disease burden every year. Successful control of these organisms by the host depends on the inflammatory cytokine Tumor Necrosis Factor (TNF). While TNF is known to protect against many intracellular bacterial infections, and the molecular mechanisms of TNF signaling are understood in sterile contexts, the precise modes through which TNF can defend the host against bacterial infection remain unclear. The following research aims to elucidate the mechanism of TNF in the context of intracellular infection. In this project, the gram-negative bacteria Legionella pneumophila, the causative agent of Legionnaire’s Disease, acts as a model intracellular pathogen. Infection of bone marrow derived macrophages and live mice are both used to explore the effect of TNF signaling on control of infection. Thus far we have shown that TNF is required for restriction of bacterial replication both in vitro and in vivo. We have also demonstrated that TNF is required for optimal production of the pro-inflammatory cytokines IL-1alpha, IL-1beta, and IL-6. In addition, the cysteine protease caspase 8 appears to be necessary for control of Legionella infection and may be the mechanism through which TNF restricts bacterial replication.
Cytokines are required for our immune system to fight pathogens. Distinct cytokines are both secreted by and required for differentiation of distinct CD4+ T Helper (Th) subpopulations (e.g. Th1 or Th17 cells), which in turn fight distinct classes of pathogens. Specifically, Th cells differentiate from naive CD4 T cells upon receiving three signals simultaneously: activation of the T cell receptor (TCR), activation of the costimulatory receptor CD28 and induction by specific cytokines. Activation of the TCR and CD28 receptors in the absence of cytokines results in differentiation to a generic Tho state. By contrast, the addition of particular cytokines activates distinct transcription factors resulting in well-studied Th-specific changes in gene transcription that drive differentiation. In previous studies we have demonstrated widespread alternative splicing upon activation of naive CD4+ T cells to Tho cells. However, the scope and role of cytokine-induced splicing as an additional layer of gene regulation during Th cell differentiation has not been explored. In this study, I identified splicing differences between in vitro differentiated Tho and Th1 or Th17 cells. My results suggest splicing may play a functionally important role during Th cell differentiation.
Human genetics has proven to be a powerful approach to identify novel therapeutic targets for coronary artery disease (CAD), the leading cause of death worldwide. Genetic variants at chromosomal locus 8q24, 40kb from the TRIB1 gene, are significantly associated with CAD, as well as with known CAD risk factors: plasma LDL and triglyceride rich lipoproteins. Hepatic overexpression of Trib1 in mice decreases plasma lipid and hepatic fat, while liver-specific Trib1 deficiency (Trib1 LSKO) increases plasma lipid, lipogenesis and hepatic steatosis. TRIB1 encodes a pseudokinase protein that targets the transcription factor CEBPα for proteosomal degradation. Our lab has shown that the increased lipogenesis and hepatic steatosis in Trib1 LSKO mice are attributable to increased protein levels of Cebpα, however, the role of Cebpα in regulating plasma lipids was unclear. My results show that Cebpα hepatic deletion alone reduces plasma lipid levels, implicating Cebpα in their regulation. However, this effect persists in combination with Trib1 LSKO, indicating that it is independent of Trib1. Furthermore, the physiological mechanism by which Trib1 regulates plasma lipids is unknown. My data shows that Trib1LSKO mice have decreased LDL clearance compared to control mice. These results suggest that Trib1 modulates plasma lipids by regulating LDL-C catabolism.
Identification of tumor-derived antigens is critical for the development of personalized immunotherapy. Neoepitope load and quality in solid tumors correlates with response to immunotherapy and patient survival. However, no consensus method exists to sort the abundance of putative vaccine targets present in most tumors by therapeutic potential. In order to comprehensively profile and prioritize tumor antigens, we developed a software package, antigen.garnish, that predicts amino acid sequence, MHC-binding affinity, and microbial homology of missense, fusion, and frameshift-derived neoepitopes as well as their wild-type counterparts. antigen.garnish predicts neoepitopes from variant calls, cDNA, or protein sequences. Seven binding prediction algorithms are integrated to produce a consensus score for MHC affinity. The program ranks epitopes by both absolute MHC I/II binding affinity and binding affinity relative to similar sequences in the normal peptidome, termed the differential agretopicity index (DAI). An overall immune fitness score then integrates neoepitope quality, variant clonality, and alignment to experimentally confirmed immunogenic microbial peptides. To demonstrate the application of antigen.garnish, we validate our method against published data, profile neoepitope load across murine cell lines, and identify top neoepitopes for vaccination.
The YAP1-NF-κB axis promotes sarcomagenesis by inactivating circadian clock-mediated unfolded protein responses and autophagy

Adrian Rivera-Reyes, Shuai Ye, Gloria Marino, Shaun Egolf, Gabrielle Ciotti, Susan Chor, Ying Liu, Jessica Posimo, Paul M. Park, Koreana Pak, Jaimarie Sostre-Colon, Feven Tameire, Nektaria M. Leli, Constantinos Koumenis, Donita Brady, Anthony Mancuso, Kri

Terminal differentiation opposes proliferation in the vast majority of tissues. Loss of lineage differentiation is a hallmark of aggressive cancers, including soft tissue sarcomas (STS). Undifferentiated Pleomorphic Sarcoma (UPS), an STS subtype devoid of lineage markers, is among the most lethal sarcomas in adults. Though tissue-specific features are lost in these mesenchymal tumors, they are most commonly diagnosed in skeletal muscle and are thought to develop from transformed muscle progenitor cells. We have found that a combination of HDAC (Vorinostat) and BET bromodomain (JQ1) inhibition partially restores differentiation to skeletal muscle UPS cells and tissues, enforcing a myoblast-like identity. Importantly, differentiation is partially contingent upon downregulation of the Hippo pathway transcriptional effector, Yes-activated protein 1 (YAP1) and its downstream effector NF-κB. Previously, we observed that Vorinostat/JQ1 suppresses YAP1 and NF-κB activity inhibiting tumorigenesis and promoting differentiation. However, the mechanisms by which the Hippo/NF-κB axis controls differentiation remained unknown. Treatment with Vorinostat/JQ1 inhibited glycolysis/MTOR signaling, activated the clock, and upregulated the UPR and autophagy via inhibition of YAP1/NF-κB. These findings support the use of epigenetic modulators to treat human UPS and define the connection between these pathways and differentiation. Additionally, we identify various genes as potential biomarkers of treatment efficacy.

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bNAbs targeting conserved epitopes in the HIV-1 Env V2 apex are frequently elicited during natural human infection and can be potent and broadly reactive despite low levels of somatic hypermutation, making them attractive vaccine candidates. We constructed seven novel simian-human immunodeficiency viruses (SHIVs) bearing primary HIV-1 Envs demonstrated to bind germline V2 bNAbs and used them to infect 33 rhesus macaques (RMs): SHIVs CH505 (n=6), CAP256SU (n=6), CH1012 (n=6), ZM233 (n=3), WITO (n=3), T250 (n=6) and Q23 (n=3). All RMs developed autologous tier-2 NAb responses (reciprocal IC50 = 0.05-0.00001) that were proportional to plasma vLoad. Twelve animals developed neutralization breadth against a subset of 20 heterologous tier-2 viruses. This included two RMs infected with SHIV-T250; two infected with SHIV-CH505; two infected with SHIV-CAP256SU; and one infected with SHIV-Q23. In these RMs, bNAb responses mapped to the C strand of V2 with variable dependence on N160 glycan and selected for varied patterns of viral escape at residues 165, 166, 168, 169 or 171. Five RMs infected by SHIVs CH1012, ZM233 or CAP256 developed bNAbs targeting other non-V2 apex epitopes. Analysis of Env-Ab coevolution in SHIV-infected RMs may serve as an important “molecular guide” for HIV vaccine design.
The tumor microenvironment (TME) is composed of different cell types including fibroblasts and endothelial cells. Cancer cells are hyperproliferative depleting resources thus subjecting normal cells in the TME to various stressors including amino acid deprivation (AAD). Cells upregulate mechanisms that allow them to tolerate stress, but in the presence of persistent stress, cells activate the integrated stress response (ISR). AAD specifically upregulates the kinase GCN2 which in turn phosphorylates the eukaryotic initiation factor alpha (eIF2α) resulting in both inhibition of global protein translation and upregulation of the transcription factor ATF4. ATF4 subsequently activates apoptosis or induces autophagy or senescence to prolong survival. Ablation of either GCN2 or ATF4 inhibits tumor growth. Studies have shown that the p53 tumor suppressor promotes survival in response to AAD by altering cellular metabolism. We are investigating the role of P19ARF in the TME as sarcoma cells injected into the flank of p19ARF/- mice show enhanced tumor growth as compared to WT mice. These data presented suggest a previously unknown role for the tumor suppressor p19ARF in fibroblasts in the TME and may offer new targets in the TME to block tumor growth and progression.
Jesse Rodriguez

GTV

**CMV-specific CAR T cells exhibit anti-tumor activity against CMV-positive glioblastoma multiforme**

Jesse L. Rodriguez, Avery D. Posey, Jr., MacLean Nasrallah, Alina C. Boesteanu, Reiss Reid, Tong Da, Sangya Agarwal, Lualhati E. Harkins, Kristin Blouch, Nadia Dahmane, Zev A. Binder, Donald O’Rourke, James C. Alwine, Marco Ruella, Laura A. Johnson, Carl H. June

Glioblastoma multiforme (GBM) is the most common and deadliest primary brain tumor. Immunotherapeutic approaches using chimeric antigen receptor (CAR) T cells have shown limited efficacy against GBM due to heterogeneous target antigen expression. Cytomegalovirus (CMV) can be detected in up to 90% of GBM tumor samples but not the surrounding normal brain tissue. We detected the presence of CMV in GBM tumor samples in approximately 77% of samples via IHC. A CAR was generated and optimized to recognize the CMV surface antigen gB. In vitro testing of the anti-gB CAR revealed activity against the U87 glioma cell line stably transduced to express gB and CMV-infected human foreskin fibroblasts (HFF) cells. In vivo, gB CARs were able to treat established GBM tumors in a xenograft mouse model. gB CAR T cells demonstrated tumor recognition against the human GBM explant, D270, both in vivo and in vitro, despite undetectable levels of antigen expression. These results suggest that CAR T cells may be effective in recognizing extremely low abundance antigens, and taken together, the results of this study show the feasibility of using gB CAR T cells as a platform to target CMV in GBM tumors to treat patients with GBM.
Trib1 controls antiviral immunity by restraining CD4 and CD8 T cell effector responses during chronic infection


During chronic infection, the magnitude of the anti-viral T cell response is determined by the balance between T cell effector (TEFF) function and T cell exhaustion. While CD8 exhaustion has been extensively characterized, how TEFF responses are regulated during chronic antigen exposure is less clear. We identify Tribbles Pseudokinase 1 (Trib1) as a central regulator of the TEFF response to chronic infection. T cell specific deletion of Trib1 restored TEFF expansion and function and reduced viral load. Additionally, Trib1 regulated both CD4 and CD8 T cell responses, providing unique insight into the regulation of CD4 help during chronic disease. Mechanistically, we identified a new regulatory axis where Trib1 binds and inhibits critical signaling complexes downstream of T cell receptor (TCR) engagement, restricting T cell activation. These data support a model of negative feedback regulation where Trib1 restrains TCR signaling and downstream function, and identify a new link between Trib1 and TEFF biology that can be targeted to improve antiviral immunity.

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Jonathan Rumley

G&E

The Wnt pathway regulates anterior sister lineage genes in the C. elegans embryo

Jonathan Rumley, Amanda Zacharias, John Murray

A fundamental step in embryonic development is patterning the body axes. Anterior-posterior axis patterning depends on the Wnt pathway, which acts through the transcription factor (TF) TCF and its coactivator beta-catenin. In the C. elegans embryo the Wnt pathway is vital for the proper specification of the majority of anterior-posterior oriented cell divisions. After each anterior-posterior cell division, the posterior cell behaves as if it receives a Wnt signal: SYS-1/beta-catenin accumulates in the nucleus of the posterior sister cell and POP-1/TCF is exported from the nucleus. In the nucleus of the anterior sister, POP-1 levels remain high, and SYS-1 levels remain low. Canonical Wnt target genes are repressed in the anterior sister, in which the majority of POP-1 is not associated with SYS-1 and/or are activated in the posterior sister, in which the majority of remaining nuclear POP-1 is associated with SYS-1. Recent reports suggest that anterior genes may be activated by POP-1 without SYS-1 and repressed by POP-1 when bound to SYS-1. What is the mechanism of this regulation? I hypothesize that POP-1 binds anterior enhancers and directly activates anterior gene expression in anterior cells and inhibits their expression in posterior cells through interactions with other TFs.

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Samantha Russell

DSRB

The guidance receptor Frazzled is intrinsically required for oocyte development independently of its ligand Netrin

Samantha A. Russell, Kaitlin M. Laws, Greg J. Bashaw

Both inside and outside of the nervous system, Frazzled (Fra) plays well-documented roles in cell migration. To explore novel roles for Fra, we examined Fra expression in the ovary. The Drosophila ovary contains strings of egg chambers, each of which is composed of a single oocyte and fifteen nurse cells encapsulated by a somatic follicle cell layer. We generated germline clones of fra, with three different loss of function alleles, all of which resulted in egg chamber degeneration. Surprisingly, we have found that the loss of Netrin does not result in any egg chamber degeneration, suggesting that Fra is acting independently of Netrin during oogenesis. We know that Fra can signal in two ways: (1) in response to its ligand Netrin, Fra affects the cytoskeleton, and (2) the Fra ICD regulates transcription independently of Netrin. In the future, we will further characterize the degeneration phenotype seen with the loss of Fra and determine whether Fra regulates transcription in the developing oocyte. Determining how Fra signals in oogenesis can then be applied to other tissues and organisms that Fra, and its vertebrate homolog DCC, are required in.
Ronnie Russell

MVP

Chimpanzee CD4 Receptor Diversity Protects against SIV Infection

Ronnie M. Russell, Frederic Bibollet-Ruche, Guillaume B.E. Stewart-Jones, Weimin Liu, YingYing Li, Andrew G. Smith, Martine Peeters, Peter D. Kwong, Paul M. Sharp, Beatrice H. Hahn

CD4, the cell surface receptor for primate lentiviruses, has been reported to be under positive selection in primates, yet no association with susceptibility to SIV infection has been established. Here we identify and characterize the function of nine endemic chimpanzee CD4 alleles. These alleles are defined by five polymorphic amino acids in the D1 domain that overlap with the binding site of the SIV envelope. We show that alone or in combination these CD4 polymorphisms are associated with protection from SIVcpz infection, both in vitro and in vivo. Two distinct mechanisms account for the resistance, involving interactions between charged residues of CD4 and Env and chimpanzee-specific CD4 N-glycans sterically clashing with the glycan shield of the viral envelope. This glycan-mediated interference was not specific to SIVcpz, as CD4 glycosylation reduced the infectivity of diverse SIV lineages. The occurrence of variable N-glycosylation positioning on CD4 throughout the primate lineage, coupled with amino acid variability, suggests that CD4 diversity might represent a recurrent mechanism during primate evolution to protect against lentiviral infection.
HIV-Associated Neurocognitive Disorders (HAND) affect 55% of HIV-infected individuals worldwide. While antiretroviral treatments have reduced the severity of HAND, the prevalence has increased due to increased life expectancy. In addition, little progress has been made in developing therapeutics to reduce the prevalence of HAND. While the major pathological manifestation of HAND is synaptodendritic damage, the full, underlying mechanism is unknown partly due to the fact that there is no in vitro model to study the direct interactions between HIV-infected macrophages/microglia and neurons. In order to address this problem, we have developed a human-induced pluripotent stem cell (HiPSC) based model; whereby, we separately differentiate HiPSCs into forebrain, glutamatergic-like neurons, astrocytes, and microglia and create a co-culture of the three cell types with or without HIV-infection. This novel, reductive system allows us to study the direct interactions and mechanisms by which microglia cause synaptodendritic damage in HAND progression.
Alexander Sakers

G&E

Identification of a mesenchymal progenitor cell hierarchy in adipose tissue

Alexander Sakers, David Merrick, Zhazira Irgebay, Chihiro Okada, Michael P. Morley, Patrick Seale

The healthy growth of adipose tissue depends on the capacity of progenitor cells to undergo de novo adipogenesis. However, the cellular hierarchy and mechanisms governing adipocyte progenitor differentiation are poorly understood. Here, we identify a lineage hierarchy consisting of three distinct mesenchymal cell types in adipose. We find that cells marked by Dpp4 are highly proliferative, multipotent progenitors that give rise to Icam1+ committed pre-adipocytes and a distinct adipogenic population marked by Clec11a and Cd142 expression. TGFβ maintains DPP4+ cell identity and inhibits adipogenic commitment of DPP4+ and CD142+ cells. Intriguingly, DPP4+ progenitors reside in the reticular interstitium that envelopes many organs including adipose depots. Altogether, this study defines the adipose lineage hierarchy and identifies a new anatomical niche for multipotent mesenchymal progenitors.

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Clear cell renal cell carcinoma (ccRCC) is a subtype of kidney cancer defined by robust lipid accumulation, which prior studies have indicated plays an important role in tumor progression. We hypothesized that the peroxisome proliferator-activated receptor gamma (PPARg), detected in both ccRCC tumors and cell lines, promotes lipid storage in ccRCC and contributes to tumorigenesis in this setting. PPARg transcriptionally regulates a number of genes involved in lipid and glucose metabolism in adipocytes, yet its role in ccRCC has not been described. Using chromatin immunoprecipitation followed by deep sequencing (ChIP-seq), we found that PPARg and its heterodimer RXR occupy the canonical DR1 PPAR binding motif at approximately 1000 locations throughout the genome that can be subdivided into adipose-shared and ccRCC-specific sites. CRISPR-Cas9 mediated, loss-of-function studies determined that PPARg is dispensable for viability, proliferation, and migration of ccRCC cells in vitro and in vivo. Also, surprisingly, PPARg deletion had little effect on the robust lipid accumulation that typifies the “clear cell” phenotype of kidney cancer. Our results suggest that PPARg plays neither a tumor suppressive nor oncogenic role in advanced ccRCC, and thus single-agent therapeutics targeting PPARg are unlikely to be effective for the treatment of this disease.
Sabine Schneider

G&E

Elucidating the Regulation of Enteric Neuron Subtype Specification and Differentiation

Sabine Schneider, Sohaib K. Hashmi, Christina M. Wright, Paul J. Gadue, Faranak Fattahi, Robert O. Heuckeroth

The enteric nervous system (ENS) is a complex network of 500 million neurons and glia that spans the length of the bowel. Imbalances in the over 20 highly specialized enteric neuron subtypes may underlie a variety of debilitating gastrointestinal motility disorders. Surprisingly, we do not understand how ENS precursors differentiate into mature neuron subtypes. We are using a recently described human embryonic stem cell-derived enteric neuron model to identify factors that drive differentiation of ENS precursors into enteric neuron subtypes. We are using single cell transcriptomics data to identify differentially expressed transcription factors and cell signaling pathways uniquely activated in enteric neuron subpopulations. We are overexpressing transcription factors and test trophic factors in our in vitro differentiation system to assess whether the ratio of enteric neuron subtypes is skewed towards specific lineages. To facilitate detection of motor neurons in our mixed cell populations, we are generating fluorescent reporter cell lines. We have identified several transcription factors that are unique to distinct murine enteric motor neuron subpopulations. Understanding the intra- and extracellular signals regulating enteric neuron differentiation may shed light on the pathogenic mechanisms underlying enteric neuropathies and pave the way for the development of cell-based therapies.

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Elisha Segrist

MVP

Investigating the Antiviral Role of STING during Enteric RNA Virus Infection in Drosophila

Elisha Segrist, Beth Gold, Sara Cherry

The intestine serves as a physical and immune barrier to protect against infection by enteric pathogens. The Cherry lab uses Drosophila as an in vivo model of human and mosquito enteric infection to understand the intestinal innate immune response and how it is influenced by the composition of the microbiota. Sindbis virus is a mosquito-borne virus that is transmitted to vectors orally during a blood meal. Our preliminary data suggest the Drosophila homologue of Stimulator of Interferon Genes (STING) is antiviral against Sindbis virus within the intestine. Mammalian STING is activated by binding cyclic dinucleotides (CDNs) produced endogenously by cyclic GMP-AMP synthase or exogenously by bacteria. Metagenomic data reveal that Drosophila commensal bacteria encode CDN synthases indicating the microbiota could provide an exogenous CDN pool to prime basal STING activity from subsequent viral infection. Indeed, our preliminary data suggests exogenous feeding of cyclic dinucleotides to flies lacking their microbiota protects against enteric Sindbis virus infection. Moreover, we found that STING is antiviral through the activation of autophagy and inflammatory NF-kB activation. This work sheds light on the ancient cGAS-STING defense pathway and defines how additional microbiota-derived products, such as CDNs, may prime intestinal immunity.
Iryna Shakhmantsir

DSRB

Spliceosome Factors Target timeless (tim) to Maintain Clock Function and Circadian Behavior in Drosophila

Iryna Shakhmantsir, Soumyashant Nayak, Gregory R. Grant, Amita Sehgal

Transcription-translation feedback loops that comprise eukaryotic circadian clocks rely upon temporal delays, which separate the phase of active transcription of clock genes, such as Drosophila period (per) and timeless (tim), from negative feedback by the two proteins. However, mechanisms underlying such delays are unclear. Through an RNA interference screen, we found that pre-mRNA processing 4 (PRP4) kinase, a component of the U4/U5,U6 triple small nuclear ribonucleoprotein (tri-snRNP) spliceosome, and other tri-snRNP components regulate cycling of the molecular clock as well as rest:activity rhythms. Unbiased RNA-Sequencing uncovered an alternatively spliced intron in tim whose increased retention upon prp4 downregulation leads to decreased TIM levels. We demonstrate that the splicing of tim is rhythmic with a phase that parallels delayed accumulation of the protein in a 24-hour cycle. We propose that alternative splicing constitutes an important clock mechanism for delaying the daily accumulation of clock proteins, and thereby negative feedback by them.
Kamen Simeonov

DSRB

Profiling the spread of metastatic colorectal cancer via CRISPR lineage tracing

Kamen Simeonov, Aaron Mckenna, Xin Wang, Zvi Cramer, Jay Shendure, Christopher Lengner

Cancer metastasis is the process by which a highly-treatable, localized lesion transforms into a systematic, incurable disease. In the case of colorectal cancer, a 90% 5-year survival rate for localized disease drops to just 14% for metastatic cancer. To improve outcomes, we must advance our understanding of (1) the path that CRC takes to metastasize across the body and (2) the molecular adaptations that allow cells to traverse this path. To address these points, we are engineering a tunable and inducible CRISPR/Cas9 lineage tracing system that enables cells to record a written history of their cell divisions into synthetic barcodes integrated in their genomic DNA. By activating CRISPR lineage tracing in a tumor prior to metastasis and subsequently sequencing cellular barcodes and transcriptomes, we will build a precision map of cancer metastasis at single cell resolution, allowing us to find vulnerabilities that can be exploited for the development of future therapies. More broadly, our method allows high-resolution tracing of any cell lineage and is thus poised to revolutionize the way in which we probe integral questions in cancer biology, development, and stem cell biology and regeneration.
Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant neurodegenerative
disease. In SCA2 there is progressive loss of coordinated movement. SCA2 is caused by
a CAG repeat expansion within ATXN2, encoding mutant ATXN2 with an expanded
polyglutamine (polyQ) tract. Cerebellar Purkinje cells and other neurons of the central
nervous system (CNS) are affected in SCA2, with neuronal dysfunction and atrophy
caused by a toxic gain-of-function mechanism. There are currently no effective
treatments for SCA2. I am developing two CRISPR-Cas9 gene editing strategies to
reduce ATXN2 expression. I have identified single-guide RNA (sgRNA) sequences that
target the endonuclease, Streptococcus pyogenes Cas9 (SpCas9) to ATXN2 and
effectively reduce its expression. CRISPR interference (CRISPRi) is an alternative
strategy that inhibits transcription by taking advantage of a deactivated
Staphylococcus aureus Cas9 (dSaCas9) coupled to a repressive domain. The CRISPR-
Cas9 systems will be delivered in vivo using recombinant adeno-associated virus (rAAV)
Vectors, with the goal of preventing or reversing disease phenotypes in SCA2 mice
models.
Gregory Sousa

MVP

The role of CLIPC9 in the melanization immune response in the malaria vector Anopheles gambiae

Gregory L. Sousa, Michael Povelones

One arm of the insect immune response, the melanization cascade, is regulated by a hierarchical network of CLIP domain serine proteases (CLIPs). CLIPs are arthropod-specific enzymes that are divided into multi-member subfamilies (CLIPA-E). Exposure to microbial patterns and/or microbial-derived proteases ultimately results in the activation of a CLIPC. Active CLIPCs typically cleave and activate members of the CLIPB family, which function as terminal proteases. Active CLIPBs then drive melanin production by activating phenoloxidase (PO), which is the rate-limiting melanization enzyme. CLIPAs are catalytically inactive but play important regulatory roles. Much of our understanding of CLIP networks comes from work done in classic, genetically or biochemically tractable insect models with considerably less detail known in mosquitoes. To address this knowledge gap, this project seeks to be the first identification of a CLIPC participating in the melanization immune response in a mosquito. We have evidence that CLIPC9 is required for the melanization of bacteria and Plasmodium parasites. Furthermore, we report that CLIPC9 silencing may alter the utilization or localization of a phenoloxidase we hypothesize is required for melanization following bacterial challenge. Mechanistic studies are on-going to understand the contributions of CLIPC9 in antimicrobial defense in this disease vector.
Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disease characterized by extra-skeletal bone formation within skeletal muscle, caused by a gain-of-function mutation in the BMP type 1 receptor ACVR1. Our data show that muscle tissue from Acvr1R206H mice after injury remains damaged and fibrotic compared to injured WT muscle that is fully restored at 14 days, indicating muscle repair is impeded by the Acvr1R206H mutation. Additionally, Acvr1R206H animals develop a substantial amount of bone within the muscle 21 days after injury. BMP signaling has been shown to promote proliferation of muscle stem cells (MuSCs) and repress their differentiation, so we hypothesize that elevated BMP signaling in Acvr1R206H MuSCs impairs their ability to regenerate muscle after injury. We isolated MuSCs via fluorescent activated cell sorting, and found no significant differences in proliferation of WT or FOP MuSCs from injured muscle. We next investigated the ability of WT and FOP MuSCs to differentiate in vitro via myogenic media. WT MuSCs fuse to form branching myofibers, but FOP MuSCs form underdeveloped fibers that fail to fuse. These data indicate that the Acvr1R206H mutation does not affect MuSC proliferation, but negatively impacts the ability of MuSCs to undergo myogenesis during skeletal muscle regeneration.
The Bone Morphogenetic Protein (BMP) pathway patterns dorsal-ventral (DV) tissues during gastrulation. A dimeric BMP ligand assembles a receptor complex composed of two type-I and two type-II receptors. Type-II receptors phosphorylate and activate type-I receptors, which then phosphorylate Smad proteins, which regulate gene expression. This, however, is overly simplistic as there are two conserved classes of type-I receptor, Bmpr1 and Acvr1, and two conserved classes of type-II receptor, Bmpr2 and Acvr2, all of which are necessary for vertebrate development. In the zebrafish embryo, Bmp2/7 heterodimers are the only ligands that signal in DV patterning. This arises from the heterodimer’s unique ability to integrate both type-I receptors into the BMP receptor complex, as Bmpr1 preferentially binds the Bmp2 ligand, and Acvr1 exclusively binds Bmp7. I hypothesize that Bmpr1 and Acvr1 have distinct functional roles. I am performing a series of domain swap experiments to determine the components required for each receptor's specific function. We do not currently know the contribution of the two BMP type-II receptor classes, Bmpr2 and Acvr2, to the signaling complex. I am creating zebrafish mutants null for each type-II receptor class using CRISPR technology, to determine whether both classes have independent, necessary signaling functions in DV patterning.
The Integrated Stress Response (ISR) transcription factor ATF4 mediates survival during MYC-induced lymphomagenesis

Feven Tameire, Ioannis I. Verginadis, Nektaria Maria Leli, Christine Polte, Crystal S. Conn, Maria A. Monroy, Weixuan Fu, Paul Wang, Andrew Kossenkov, Jiangbin Ye, Zoya Ignatova, Serge Y. Fuchs, J. Alan Diehl, Davide Ruggero, Constantinos Koumenis

The proto-oncogene MYC is often deregulated in human tumors and particularly in lymphomas, its overexpression associates with poor prognosis. ISR kinases, PERK and GCN2, phosphorylate eIF2α, transiently inhibiting protein translation during times of stress. p-eIF2α promotes the preferential translation of the transcription factor ATF4, which activates a gene expression program to promote recovery from nutrient deprivation and ER stress. Here, we demonstrate the critical role ATF4 plays in promoting the survival of MYC overexpressing cells both in vitro and in vivo. In vitro, ATF4 depletion significantly sensitized cells to apoptosis following MYC activation. MYC activation leads to significant accumulation of uncharged tRNAs which led to activation of GCN2 in RNA Polymerase III dependent manner. ChIP-seq analysis of ATF4 and MYC identified gene targets that MYC and ATF4 co-occupied which showed enrichment for pathways involved in tRNA charging, amino acid transport and biosynthesis. Consistent with our in vitro data, we observe higher levels of p-eIF2α and ATF4 in lymphoma cells in a mouse model of MYC driven lymphoma. Importantly, excision of ATF4 significantly delayed MYC-driven lymphomagenesis and promoted survival of lymphoma bearing mice. Our findings establish that ATF4 cooperates with MYC in regulating pro-survival pathways supporting MYC induced lymphomagenesis.
Jayesh Tandel

MVP

Understanding the transcriptional development of sexual stages of Cryptosporidium - An important pre-requisite for unravelling the programming of the life cycle of the parasite

Jayesh V. Tandel, Adam Sateriale, Brittain Pinkston, Meghan Sullivan, Daniel Beiting, Carrie Brooks, Boris Striepen

Cryptosporidium is the second major leading cause of diarrhea-induced mortality in infants after rotavirus. Currently, there are no drugs or vaccines against the parasite, and the understanding of the parasite biology is scant. Cryptosporidium infection is initiated by ingestion of oocysts. Sporozoites emerge from the oocysts and then infect intestinal epithelial cells. Then parasites undergo asexual replication, sexual development and sex to produce oocysts. Oocysts can autoinfect the same host or can be shed through feces for transmission. Currently, the mechanism of maintaining chronicity of infection is not known. There are two different models that can explain the persistence of infection. The first model suggests that Cryptosporidium replicates asexually and undergoes gametogenesis concomitantly (Model A). Alternatively, the parasite might undergo limited rounds of asexual replication followed by obligatory gametogenesis (Model B). If the latter is true, then it is possible to block parasite amplification and transmission by preventing the development of sexual stages into oocysts. Our preliminary in vitro data suggest that the asexual population completely differentiates into sexual stages which indirectly support the model of obligatory sexual development to maintain infection in a host. We aim to test this model by first defining transcriptome of sexual stages.
Investigating the functional role of mutant p53 in esophageal cancer related lung metastasis

Qiaosi Tang, Ashley A. Lento, Tatiana Karakasheva, Veronique Giroux, Jinyang Li, Taiji Yamazoe, Ben Z. Stanger, Maureen E. Murphy, Anil K. Rustgi

Esophageal squamous cell carcinoma (ESCC) is a lethal cancer worldwide. ESCC frequently metastasizes to lung, which leads to a poor prognosis. TP53 mutation is the most frequent genetic alteration in ESCC, and p53R175H (homologous to Trp53R172H in mice) is a common hot spot mutation. How metastasis is regulated by p53R175H in ESCC remains to be elucidated. To investigate p53R175H in ESCC, our lab utilized germline Trp53R172H/- mice, and generated esophageal specific Trp53/- mice and Trp53+/+ mice treated with the 4-NQO carcinogen to model ESCC. In the primary Trp53R172H/- tumor cell lines established from these mouse models, we depleted Trp53R172H and observed a reduction in cell invasion in vitro and subcutaneous tumor growth in vivo. We are now using tail-vein injection to examine the effects of mouse esophageal cancer cell lines Trp53R172H upon lung metastasis. Furthermore, we will perform RNA-seq to compare gene expression profiles of metastatic Trp53R172H/- and Trp53/- cells to identify genes and pathways that are significantly altered between Trp53R172H/- and Trp53/- cells metastatic cells. Drugable targets will be identified. Drug testing experiments in metastatic Trp53R172H/- and Trp53/- cell 3D organoids will be performed. Taken together, this study will improve our understanding of mutant p53 oncogenic effects in ESCC metastasis.
DDX56 is antiviral against alphaviruses and binds Chikungunya virus RNA during infection

Frances Taschuk, Sara Cherry

Chikungunya virus (CHIKV) is an emerging arthropod-borne virus in the alphavirus group, and causes outbreaks around the world. We performed an RNAi screen and found that the nucleolar helicase DDX56 has an antiviral role in alphavirus infection. We have found that DDX56 is antiviral against Chikungunya virus, as well as the related alphaviruses Sindbis and O’Nyong’Nyong. Alphaviruses are known to be controlled by type I interferons, but we found that DDX56 is dispensable for interferon signaling. Therefore, we hypothesized that DDX56 exerts its antiviral activity through binding to viral RNA. We used CLIP-Seq of endogenous DDX56 to identify interacting RNAs, and found that a region of the CHIKV genome near the 5’ end of nsP4 is bound by DDX56. While nsP4 is produced as part of a polyprotein (nsP1-4), its levels are regulated by degradation of free nsP4 and, in some alphaviruses, by readthrough of an opal stop codon at the end of nsP3. The region we identified is just downstream of nsP3, suggesting that DDX56 attenuates CHIKV infection by interfering with translation of nsP4, and we are currently defining the mechanism by which DDX56 binding to this region of the genome impacts viral protein expression and replication.
HIV integrase (IN), the enzyme that catalyzes viral integration into genomic DNA, is targeted by inhibitors that bind the IN active site. A second class of compounds, the allosteric integrase inhibitors (ALLINIs), bind a different site and act by a different mechanism. ALLINIs have not yet been implemented clinically, pending improvements in specificity, potency, and barriers to resistance -- goals that can be achieved through a better understanding of the structural mechanism of action of ALLINIs. Previously, we co-crystallized full-length HIV IN with an ALLINI and determined the structure of this complex. We have determined additional structures of other ALLINIs and IN proteins containing resistance substitutions. The structures reveal the formation of a polymer of IN, consisting of contacts between the catalytic core domain and C-terminal domain, centered around the ALLINI. The contacting surfaces are rich in residues that convey resistance to ALLINIs, as identified by serial viral passage experiments. During virion production, ALLINIs induce the formation of IN polymers, thereby aggregating and sequestering IN, and ultimately resulting in dysfunctional, non-infectious virions. Our structures reveal structural mechanisms of resistance to ALLINIs and new structures offer a route toward improved characterization of the drug binding interface.
Regulation of phalangeal joint development by ACVR1 in fibrodysplasia ossificans progressiva

O. Will Towler, Frederick S. Kaplan, Eileen M. Shore

Fibrodysplasia ossificans progressiva (FOP; MIM #135100) is a genetic disease of heterotopic ossification accompanied by short, laterally deviated great toes, caused by an activating mutation in the BMP receptor ACVR1/ALK2. To investigate the skeletal developmental phenotype of FOP, we used a conditional knock-in mouse model (MGI:5763014) with the FOP ACVR1 R206H mutation to examine digit and joint formation. Mice expressing the FOP mutation globally or in limb mesenchymal cells (Prrx1+) exhibited stunted hindlimb first digits similar to FOP patients and revealed generalized delayed digit development. Three-dimensional whole-mount pSmad1/5 immunohistochemistry and imaging of embryonic mouse limbs revealed unrestricted BMP signaling throughout the developing digit rays of mutant animals, supporting that BMP pathway activity from the mutant receptor was insufficiently inhibited in the joint space. Expressing the mutation only in joint progenitor cells using Gdf5-Cre showed that mutation activity in these cells was sufficient to induce the joint and digit phenotype. Histological analyses revealed disorganized, interphalangeal chondrocyte proliferation in both Prrx1-Cre and Gdf5-Cre models, supporting the hypothesis that the phenotype is due to improper spatiotemporal activation of chondrogenic pathway signaling in the joint space that is precipitated by increased BMP pathway signaling through the mutant ACVR1 receptor during skeletal development.
Mutations in the Crumbs homologue 1 (CRB1) gene account for 10-15% of all Leber’s congenital amaurosis (LCA) cases. Understanding the disease pathology is fundamental to therapeutic development. However, in vivo functional studies of CRB1 are complicated by the differential expression pattern of CRB1 between human and murine retinas. To investigate the early stages of pathophysiology, we attempted to generate a humanized model of CRB1-associated LCA by differentiating two iPSC lines from CRB1-associated LCA patients and three healthy-sighted controls to retinal progenitor cells (RPCs) using retinal-specific conditions and collecting samples at various time points to assess expression of RPC markers and developmental signaling pathways. We achieved adequate differentiation of RPCs from patient-derived iPSC lines characterized by the temporally appropriate expression of eye field transcription factors necessary for development of retinal cell lineages. Differences in the number of proliferating cells between CRB1-associated LCA and control RPCs were observed to occur during the crucial transition from neuroepithelium to RPC and may partially be explained by alterations in the expression of Wnt signaling pathway components.
A universal requirement of developing nervous systems is the ability for axons to find their correct synaptic targets. Developing neurons deploy axons, whose tips consist of dynamic growth cones bearing axon guidance receptor molecules that signal attraction or repulsion in response to extracellular cues. During Drosophila embryonic development, the conserved attractive axon guidance receptor Frazzled (Fra) signals canonically in response to ligand binding in order to effect cytoskeletal changes that result in the axonal growth cone navigating towards the source of ligand. Our lab has shown that Fra also has a noncanonical signalling activity whereby Fra is cleaved, and the intracellular domain (ICD), translocates to the nucleus and activates transcription. However, only one transcriptional target of the Fra ICD is known. In order to uncover more Fra ICD transcriptional targets, we performed RNA-sequencing on subsets of wild-type and Fra-deficient FACS-isolated commissural neurons at a stage in embryonic development where the neurons extend their axons across the midline. Here, we report transcripts which are differentially expressed in Fra-deficient developing commissural neurons, and demonstrate how these putative Fra ICD transcriptional targets will be validated.
A genetic-based vaccine overcomes maternal antibody inhibition of immune responses

Elinor Willis, Norbert Pardi, Kaela Parkhouse, Drew Weissman, Scott E. Hensley

Infants are particularly vulnerable to infections and severe disease, including from influenza virus. One increasingly promising strategy to protect them during this period is through maternally derived immunity transferred to the neonate. Maternal antibodies (matAb) can protect the infant soon after transfer but wane over time, leaving the infant vulnerable again. Therefore, active immunity via vaccination must also be generated in the infant. Here, using a mouse model we show that influenza virus-specific matAb inhibit the development of infant antibody responses after infection with live influenza virus or vaccination with inactivated virus. However, a novel mRNA-based vaccine expressing an influenza virus protein was able to generate strong antibody responses in mice that possessed influenza virus-specific matAb, leading to long-lasting protection. This vaccine overcomes matAb inhibition through long-term antigen expression and sustained germinal center activity and does not require cell-surface antigen expression. Together, these results suggest that genetic vaccines can overcome matAb inhibition and elicit potent immune responses in infants.
Rebecca Windmueller

DSRB

Examining discrepancies in proliferative competency between mononucleated and binucleated cardiomyocytes

Rebecca Windmueller, Apoorva Babu, Michael Morley, William Zacharias, Aoi Wakabayashi, Edward E. Morrisey

Myocardial injury results in loss of cardiomyocytes (CMs). The heart’s inability to replace these cells is associated with heart failure. A potential therapeutic strategy would be to reactivate proliferation of endogenous CMs. This has proven challenging, as mammalian CMs lose competency to respond robustly to pro-proliferative stimuli following a neonatal maturation period. However, we and others have observed that mononucleated (MoNuc) CMs are substantially more responsive to pro-proliferative stimuli than are binucleated (BiNuc) CMs. Despite this observation, it remains unclear what the molecular and cellular differences are between these two CM subsets. A better understanding of the differences between these cells could provide critical insight into what determines a CM’s competency to respond to proliferative stimuli. To characterize differences between these populations we devised a novel method to obtain populations of MoNuc and BiNuc CMs by fluorescence associated cell sorting (FACS). We used this method to separate and profile the transcripational differences between MoNuc and BiNuc CMs at four timepoints spanning CM maturation. Transcriptome analysis revealed an underlying biological signature that defines the differences between MoNuc and BiNuc CMs. Furthermore, our data points to a central role for Rb in establishing divergence and maintaining differences between the two populations.

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Approximately 15% of cancers may be attributable to viral infections. Epstein-Barr virus (EBV) is a gammaherpesvirus that causes infectious mononucleosis and has been linked to multiple lymphomas and carcinomas, including Burkitt lymphoma, Hodgkin’s lymphoma, nasopharyngeal carcinoma, and gastric carcinoma. Vaccines are approved against hepatitis B and human papilloma virus, preventing infections that lead to liver, cervical, and other cancers. However, there are no prophylactic or therapeutic treatments against EBV, and over 90% of the global adult population is chronically infected. Glycoproteins at the viral surface are potential targets for prophylactic vaccines. Antibodies that bind them can neutralize the virus to prevent initial infection and the establishment of viral latency. DNA plasmids containing EBV proteins were electroporated into mouse muscle tissue and elicited immune responses to these antigens. Robust T cell responses were seen in response to vaccination, and antibodies inhibited B cell transformation. Additionally, proteins that regulate the latent cycle of EBV are expressed in the cancers that it causes. These have been used as targets for therapeutic vaccines that inhibit tumor growth in mouse models. These vaccines have the potential to prevent and treat over 200,000 cases of cancer a year.
Hong Xie, Chih-Hang Anthony Tang, Jun H. Song, Anthony Mancuso, Juan R. Del Valle, Jin Cao, Yan Xiang, Chi V. Dang, Roy Lan, Danielle J. Sanchez, Brian Keith, Chih-Chi Andrew Hu, M. Celeste Simon

Myc activation is a primary oncogenic event in many human cancers; however, these transcription factors are difficult to inhibit pharmacologically, suggesting that Myc-dependent downstream effectors may be more tractable therapeutic targets. Here, we show that Myc overexpression induces endoplasmic reticulum (ER) stress and engages the inositol-requiring enzyme 1α (IRE1α)/X-box binding protein 1 (XBP1) pathway through multiple molecular mechanisms in a variety of c-Myc- and N-Myc-dependent cancers. In particular, Myc-overexpressing cells require IRE1α/XBP1 signalling for sustained growth and survival in vitro and in vivo, dependent on elevated stearoyl-CoA-desaturase 1 (SCD1) activity. Pharmacological and genetic XBP1 inhibition induces Myc-dependent apoptosis, which is alleviated by exogenous unsaturated fatty acids. Of note, SCD1 inhibition phenocopies IRE1α RNase activity suppression in vivo. Furthermore, IRE1α inhibition enhances the cytotoxic effects of standard chemotherapy drugs used to treat c-Myc–overexpressing Burkitt’s lymphoma, suggesting that inhibiting the IRE1α/XBP1 pathway is a useful general strategy for treatment of Myc-driven cancers.
Caiyue Xu

CB

Investigation of SirT1 loss during aging

Caiyue Xu, Zhixun Dou, Shelley L. Berger

Aging is the number-one risk factor of most chronic diseases, including cancer, Alzheimer’s Disease and heart diseases. SirT1, a nuclear NAD+-dependent deacetylase, is a critical regulator of aging and age-related pathologies. Our study suggest that SirT1 protein levels are dramatically reduced in senescent cells and aged mouse tissues. However, little is known regarding the underlying mechanism. Here, we show that SirT1 is degraded through a macroautophagy pathway (hereafter as autophagy) in the nucleus during cellular senescence and aging. We discovered that SirT1 undergoes autophago-lysosomal degradation during senescence, and its decrease with age can be inhibited upon autophagy blockage. Moreover, we found that SirT1 interacts with autophagy protein LC3, and we have identified the critical region for the interaction. Our results provide insights into a previously unknown mechanism for SirT1 homeostasis, and suggest maintenance of SirT1 protein level as a new methodology to promote healthy aging.

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Mark Yarmarkovich

CB

**Discovery of novel MHC antigens in neuroblastoma and development of pMHC directed CAR T cells**

Mark Yarmarkovich, Moreno Di Marco, Tiffany Noel, Son Nguyen, Wei Li, Dimiter Dimitrov, Stefan Stevanovic, John Maris

Due to low MHC expression and low somatic mutational burden, neuroblastoma has been largely been considered a poor candidate for adoptive immunotherapy directed at MHC-presented antigens. To identify tumor-specific antigens presented by MHC, we first purified MHC from tumor cells and eluted peptides in 16 neuroblastoma tumors. We then performed LC/MS/MS on eluted peptides (ligandomics), combined these data with RNA-sequencing data from 153 neuroblastoma tumors and 1643 normal tissues, predicted pMHC binding, and identified 265 tumor-specific antigens that are not observed in healthy tissue and predicted have strong binding affinity to be recognized as T cell epitopes. We prioritized 6 of these for preclinical development for both CAR T cells and engineered TCR receptors. Using single-cell sequencing of antigen-specific CD8 cells, we have cloned TCRs against these antigens, and also generating CAR T receptors against the pMHC using phage display. Preliminary data shows that primary CD8 cells transduced with CAR T constructs against pMHC tumors antigens have potent killing against neuroblastoma cells. However, we have observed non-specific interactions between the CAR scFv and the MHC. Ongoing work is directed at abrogating the CAR specificity against MHC using a combination of mutagenesis and structural biology.
Autophagy maintains cellular homeostasis by degrading and recycling cytosolic components in lysosomes. Three main types of autophagy exist in most mammalian cells, namely macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). Different from other two types of autophagy, chaperone-mediated autophagy selectively targets only cytosolic proteins for lysosomal degradation and doesn’t involve in the formation of vesicles. AMPK serves as the major cellular energy sensor. While the role of AMPK in activating macroautophagy has been deeply studied, whether AMPK regulates chaperone-mediated autophagy pathway is totally unknown. Here, by utilizing a photoactivatable reporter system, we found that AMPK could suppress CMA pathway. Moreover, we've shown that the inhibition of CMA pathway by AMPK is independent of Reactive Oxygen Species (ROS) level, mTOR signalling and macroautophagy. CMA activity is dependent on the level of LAMP2A on the lysosomes. Interestingly, knockdown of AMPK leads to accumulation of LAMP2A and oligomerization on the lysosomes, which is caused by decreased cathepsin A activity. Taken together, our study provides a potential pathway that how AMPK suppresses chaperone-mediated autophagy.
Global regulation of H3K36me2 underlies perturbations in epithelial plasticity

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Metastasis and chemoresistance are associated with a form of cellular plasticity known as the epithelial-to-mesenchymal transition (EMT). Though EMT has been widely associated with the activity of transcription factors, there is increasing evidence that epigenetic modifiers also play a role. Still, the true extent to which EMT depends on epigenetic mechanisms remains largely unexplored. We therefore performed a targeted CRISPR screen in plastic pancreatic cancer cells to unbiasedly identify epigenetic modifiers critical for the induction and/or maintenance of the epithelial or mesenchymal states. This resulted in several candidates of interest, including those involved in the writing, erasing, and reading of the histone post-translational modification H3K36me2. More specifically, subsequent studies have confirmed the necessity of Nsd2, a writer of H3K36me2, in the induction of the mesenchymal state, while Kdm2a, an eraser of H3K36me2, was found to be important for the epithelial state. These genes were also found to have functional implications in invasion and metastasis. Furthermore, we find that global levels of H3K36me2 is itself both a marker and regulator of EMT. These findings demonstrate a novel role for H3K36me2 as a central regulator of EMT, thereby also implicating a novel mechanism by which global epigenomic changes underpin epithelial plasticity.
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DSRB

Regulation of the non-canonical Frazzled axon guidance pathway during midline crossing in Drosophila

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It is recently discovered that the conserved Frazzled (Fra)/DCC receptor promotes midline attraction through a novel non-canonical pathway. In this pathway, Fra is cleaved to form a transcriptionally active intracellular domain that activate gene expression. However, it remains unexplored which upstream genes are required to regulate the proteolytic cleavage of Fra to allow the precise spatial and temporal control of Fra transcriptional activity. In this study we aim to identify the ligand and the ADAM metalloprotease that function in the non-canonical Fra pathway. Our genetic and biochemical experiments suggested that additional Fra interacting proteins are present at the midline prior to midline crossing. Our current experiments aim to identify the non-canonical Fra ligand via affinity pull down coupled with mass spectrometry. To identify the metalloprotease, we first showed the ADAM metalloprotease Tace is expressed in both early stage embryos and Drosophila S2R+ cells. In addition, genetic interaction experiment suggested that Tace promotes midline attraction. Future efforts will focus on further characterizing the function of Tace in the non-canonical Fra pathway. This study will define the previously unexplored upstream regulatory events of the non-canonical Fra pathway, with the potential to reveal a unifying proteolytic mechanism for all axon guidance receptors.