Salk T32 Cancer Day Symposium

Friday, December 14, 2018
Salk Institute for Biological Studies
La Jolla, California

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Salk T32 Cancer Symposium 2018: Tumor Progression and Metastasis

Salk Institute for Biological Studies

Friday, December 14, 2018

Schedule of Events

8:00am - 9:00am	Registration Check-in & Breakfast
9:00am - 9:10am	Welcome and Opening Remarks by Jovylyn Gatchalian
9:10am – 9:55am	Lewis Chodosh, University of Pennsylvania **"Preventing Metastatic Recurrence by Targeting Tumor Dormancy"**
9:55am - 10:10am	Tomoaki Hishida*, Salk Institute **"Myc Induces Dedifferentiation to Tumor-initiating Cells in Stomach"**
10:10am - 10:25am	Jovylyn Gatchalian*, Salk Institute **"BAF Complex Heterogeneity in Development and Disease"**
10:25am – 10:35am	*Coffee Break*
10:35am - 11:20am	Heide Ford, University of Colorado Denver **"Developing Novel Means to Inhibit Metastasis through Targeting Tumor Heterogeneity and EMT"**
11:20am - 11:35am	Thomas McFall*, Salk Institute **"A Systems Basis for KRAS Mutant Allele Specific Responses to Targeted Therapy"**
11:35am - 11:50am	Danna Arellano-Rodriguez*, CICESE **"CD8+ T Cells Increase Osteolytic Bone Metastases from Breast Cancer in Mice"**
11:50am - 1:20pm	*Lunch*
1:20pm - 2:05pm	Jing Yang, UCSD **"Epithelial-Mesenchymal Plasticity in Carcinoma Metastasis"**
2:05pm – 2:20pm	Kathleen DelGiorno*, Salk Institute **"Tuft Cells Play a Suppressive Role in Pancreatic Injury and Tumorigenesis"**
2:20pm - 2:35pm	Nikki Lytle*, Salk Institute **"A Multiscale Map of the Stem Cell State in Pancreatic Cancer"**
2:35pm - 3:20pm	David Tuveson, Cold Spring Harbor Laboratory, NY **"Pancreatic Cancer Science and Medicine"**
3:20pm - 3:30pm	Closing Remarks
3:30pm - 5:00pm	*Poster Session & Reception*

Invited Speaker, 30+15
*Short talk Speaker,10+5

Speaker Abstracts

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Preventing Metastatic Recurrence by Targeting Tumor Dormancy

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Despite early detection and adjuvant therapy, breast cancer remains the leading cause of cancer mortality in women, principally due to distant, incurable recurrences that arise years, or even decades, after treatment of the primary tumor. Recurrent, metastatic tumors arise from the pool of residual local and disseminated tumor cells (DTCs) that survive primary treatment in tissues throughout the body where they persist in a presumed dormant state. Importantly, women who harbor DTCs in their bone marrow have a substantially increased risk of distant recurrence, as well as poorer breast cancer-specific and overall survival. Since DTCs constitute the reservoir from which recurrent cancers arise, understanding their biology is a critical priority in cancer research. At present, however, the mechanisms enabling these cells to survive in a dormant state and ultimately recur are poorly understood and clinical methods to detect DTCs, as well as DTC-directed therapeutic approaches, are still in their infancy. To address these critical gaps, we have developed mouse models for human breast cancer that recapitulate key features of human cancer progression, including metastasis, residual disease, dormancy and recurrence. Genetic and pharmacological analysis of these models has identified several mediators of dormant DTC survival and recurrence, demonstrated their relevance to pathways that contribute to therapeutic resistance in human breast cancers, and revealed that pharmacological targets for dormancy and recurrence may be unique to these stages of tumor progression. Furthermore, we have worked on developing novel tools to enable the detection and characterization of DTCs in patients, both as a biomarker for recurrence risk and a pharmacodynamic marker to monitor the effect of therapies targeted against this population of cells. These findings and tools have, in turn, led to the initiation of clinical trials to test the ability of targeted therapies to deplete the burden of DTCs in the bone marrow of breast cancer patients and thereby decrease risk of relapse. If successful, the ability to therapeutically target DTCs would constitute a powerful new approach to preventing cancer recurrence and its associated mortality.

Myc Induces Dedifferentiation to Tumor-initiating Cells in Stomach

Tomoaki Hishida, Eric Vazquez-Ferrer, Yuriko Hishida-Nozaki, Fumiyuki Hatanaka, Javier Prieto Martinez, Reuben J. Shaw, and Juan Carlos Izpisua Belmonte
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It has been recognized that aggressiveness of cancer cells is associated with an undifferentiated state in some cases where expression of pluripotency factors, such as Oct-3/4 and Sox2, is up-regulated. It has also been believed that adult/tissue stem cells/progenitor cells (ASCs/PCs) can turn into tumor-initiating cells (TICs), indicating they should be eliminated as candidates for cancer therapy because TICs are believed to be responsible for cancer recurrence. Myc, one of the well-characterized oncogenes, is known to participate in maintaining stemness of TICs. However, it remains totally elusive whether ASCs/PCs can convert into TICs *in vivo*, and whether and how Myc directly controls TIC characteristics *in vivo*. Our results show that a gastric stem cell population can be a TIC origin. Using the murine models expressing tetracycline-inducible Myc (iMyc) together with p53 inactivation specifically in a stem cell population of the stomach, we developed metastatic gastric cancers *in vivo*. The cancer cells could be derived in a dish from the mice as serially transplantable TICs (iMyc-TICs). These TIC lines could be expanded with doxycycline (Dox), a tetracycline derivative, which can activate iMyc, while Dox withdrawal dramatically reduced their proliferative capacity, showing Myc-dependent proliferation. Interestingly, we found mutations in Ttk gene, which is frequently mutated in human gastric cancer patients and forced expression of wild-type Ttk strongly inhibited cell proliferation, suggesting that Ttk may be a critical barrier in gastric cancers. Lastly, we found that some epigenetic modifiers play some roles in maintaining TIC characteristics as Myc downstream genes. Taken together we indicate that Myc induces dedifferentiation to TIC state in stomach, which will lead to the development of Myc-targeting based innovative interventions for gastric cancers.

BAF Complex Heterogeneity in Development and Disease

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Genes encoding the subunits of the mammalian BRG1-associated factors (BAF) complex are mutated in over 20% of human cancers, but the individual contribution of each subunit in complex targeting and function remains to be defined. Here we find that the Bromodomain containing protein 9 (BRD9) and Glioma tumor suppressor candidate region gene 1 (GLTSCR1) or its paralog GLTSCR1-like (GLTSCR1L) define a smaller, non-canonical BAF complex (GBAF complex) that is distinct from the canonical BAF and the polybromo-associated BAF (PBAF) complexes. To understand the GBAF complex's localization and function in a normal setting, we used mouse embryonic stem cells (ESCs), wherein we show that GBAF and esBAF are targeted to different genomic features, with GBAF co-localizing with key regulators of naïve pluripotency. This is consistent with its specific function in maintaining naïve pluripotency gene expression, including Nanog and Prdm14. Additionally, we show that BRD9 is displaced from chromatin by the selective BRD9 bromodomain inhibitor, I-BRD9, and that this leads to changes in target gene expression. The function of the GBAF complex in ESCs is highly correlated with the function of BRD4, consistent with an association between GBAF complexes and BRD4. We find that GBAF complexes are directly recruited to chromatin by BRD4 in a bromodomain-dependent fashion and we identify a set of I-BRD9- and JQ1-sensitive BRD9 binding sites that determine the functional similarity between these epigenetic regulators. Together, our results demonstrate functionally specific roles for BAF complex assemblies in maintaining the transcriptional network of pluripotency, highlighting the biological importance of complex heterogeneity. More broadly, these studies are readily applicable to stem-like programs coopted in cancer cells and could provide a potential actionable target due to small molecule inhibitors available for BRD9.

Developing Novel Means to Inhibit Metastasis through Targeting Tumor Heterogeneity and EMT

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The role of epithelial to mesenchymal transition (EMT) in carcinomas has been controversial particularly as recent fate mapping studies have concluded that it is not critical for metastasis. However, these studies did not examine an important potential effect of EMT; that of crosstalk between epithelial and EMT cells within a heterogenous tumor. We find that cells that have undergone an EMT can increase the aggressiveness and metastatic capacity of neighboring epithelial tumor cells, both *in vitro* and *in vivo*, and demonstrate that activation of this metastatic program occurs largely through EMT cell induced non-canonical activation of GLI1 in epithelial tumor cells. We further demonstrate that inhibition of GLI directly, rather than inhibition of upstream elements of the Hedgehog (Hh) pathway, is more efficacious in preventing tumor progression in PDX models of breast cancer. However, our mouse models demonstrate that this mode of inhibition largely targets the epithelial tumor cells that are influenced by the EMT cells, as opposed to the EMT cells themselves. To target both the crosstalk between EMT and epithelial tumor cells, as well as to target the EMT cells directly, we have developed a novel small molecule inhibitor that disrupts the function of a central transcription factor in this process, Six1. This novel inhibitor targets the interaction between Six1 and a key transcriptional cofactor, Eya2, and reduces Six1 induced EMT as well as metastasis *in vivo*. The development of this novel inhibitor, as well as other means to target crosstalk between EMT and epithelial tumor cells will be discussed.

A Systems Basis for KRAS Mutant Allele Specific Responses to Targeted Therapy

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Standard protocol to treat Colorectal Cancer (CRC) includes surgery, chemotherapy and radiotherapy as first line treatment options. When applicable, targeting agents such as anti-EGFR therapies (i.e. Cetuximab and Panitumumab) have become important long-term neo-adjuvant therapies that increase survival in some patients. In recent years it has become common practice to sequence patients and their tumor specimen's DNA. This recent push in genomic medicine has led to the identification that 30-40% of CRC patient harbor an activating KRAS mutation. These mutations are thought to be an early driver in the cancer's progression. KRAS is also considered to be the key player in conferring resistance to the anti-EGFR treatments cetuximab and panitumumab. In contrast with the current guidelines for managing CRC with EGFR inhibitors, it has been shown that patients harboring a KRAS G13D mutation are sensitive to Cetuximab. Whether KRAS G13D mutations are sensitive to cetuximab has remained a controversial topic for many years due to the lack of understanding of a mechanism as it is counterintuitive to the current understanding of EGFR signaling cascade. We here identify a novel mechanism by which G13D RAS mutant is sensitive to EGFR inhibition by a non-intuitive process of reliance upon WT RAS molecules. We utilized a computational model of RAS signaling previously developed by our laboratory to explore mutant Ras signaling and thereby investigate the controversial response of KRAS G13D to anti- EGFR agents. Our computational studies of the historically reported biochemical processes that regulate Ras signals reveal a non-intuitive, mutant-specific, dependency of wild-type RAS activation on EGFR. The model also reveals this dependency is determined by the interaction strength between a KRAS mutant and tumor suppressor neurofibromin. Overall, our work demonstrates how systems approaches enable mechanism-based inference in genomic medicine.

$CD8^+$ T Cells Increase Osteolytic Bone Metastases from Breast Cancer in Mice

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Bone metastases are incurable, and we need to explore new treatments. Immunotherapy to activate T cells against cancer cells has led to promising advances. However, a potential limiting factor for its application in bone metastases is the ability for T cells to increase bone resorption, which would fuel the vicious cycle of bone metastases. To characterize the effect of T cells on bone metastases, we used a syngeneic mouse model using 4T1 breast cancer cells. When inoculated in immunocompetent Balb/C mice, 4T1 caused osteolysis detected on x-rays ($5.3\pm1.3mm^2$). When inoculated in Balb/C SCID mice lacking T and B cells, 4T1 caused less osteolysis ($1.5\pm0.3mm^2$). Histology confirmed that mice with a functional immune system had an increased tumor burden and number of osteoclast at the tumor/bone interface. Using treatment with antibodies, mice depleted of their T cells had 38% less osteolysis compared to control group. To characterize the effect of T cells on osteoclasts, $CD3^+$ T cells were isolated from the bone marrow of mice with bone metastases. In contrast with *in vivo* data, T cells from bone metastases suppressed osteoclast formation *ex vivo*. However, this effect was due to the *ex vivo* activation of T cells, consistent with decreased levels of *Rankl* and increased expression of anti-osteoclastic *Ifng* and *Il4* mRNA in activated T cells. When gene expression was measured in T cells *in vivo*, *Ifng* was not detected, and levels of pro-osteoclastic *Rankl* and *Tnfa* were higher in T cells from 4T1 bone metastases when compared to splenic T cells, suggesting that T cells in bone were not activated. Accordingly, in the bone metastases of mice, there was an increase of MDSC known to suppress T cells. 87% of the monocytic MDSC were PD-$L1^{+,}$ and could then suppress the activation of PD-1^+ T cells (>70% in bone metastases).These results suggest that unactivated T cells increase bone metastases and their activation by immunotherapy could be used for the treatment of bone metastases.

Epithelial-Mesenchymal Plasticity in Carcinoma Metastasis

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During metastasis, epithelial tumor cells dissociate from each other, disseminate into the systemic circulation, and then establish secondary tumors in distant sites. A developmental program termed Epithelial-Mesenchymal Transition (EMT) is implicated in promoting the dissemination of single carcinoma cells during metastasis. Both the Twist and Snail families of transcription factors are key inducers of EMT and tumor metastasis. Using an inducible Twist1 mouse model, we show that activation of Twist1 is sufficient to promote carcinoma cells to undergo EMT and disseminate into blood circulation. Importantly, in distant sites, turning off Twist1 to allow reversion of EMT is essential for disseminated tumor cells to proliferate and form macrometastases. These data indicate that EMT is dynamically regulated during tumor metastasis: carcinoma cells undergo EMT to disseminate; once reaching distant site, they need to revert to an epithelial identity to form macrometastases. I will also present our ongoing studies that aim to understand how EMT is dynamically regulated in response to signals from the tumor microenvironment and from the intracellular machineries to impact EMT and tumor metastasis.

Tuft Cells Play a Suppressive Role in Pancreatic Injury and Tumorigenesis

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Despite numerous advances in our understanding of pancreatic ductal adenocarcinoma (PDA) genetics and biology, this disease is expected to become the second leading cause of cancer-related deaths in the U.S. by 2020. This reflects our inability to fully understand how this disease arises, which precludes development of the early detection and interception strategies that could profoundly improve therapeutic outcomes. Acinar to ductal metaplasia (ADM) is an early event in pancreatic injury and tumor formation predicted to function in tissue healing, however the function of this elaborate cell state switching event remains unknown. Using an array of microscopy techniques, we have found that acinar to tuft cell transdifferentiation is characteristic of pancreatic metaplasia under conditions of injury or oncogenic mutation. Tuft cells are solitary chemosensory cells found throughout the hollow organs of the respiratory and digestive tracts. Their expression of taste, neuronal, and inflammatory cell signaling factors is thought to enable monitoring of intraluminal homeostasis and local response via effectors. To date, any contributions to pancreatic injury and tumorigenesis remain unknown. Here, we address these deficiencies using a combination of new strategies for tuft cell isolation, low cell number deep RNA-sequencing, metabolic profiling, and new animal models that enabled us to study the impact of selective removal of pancreatic tuft cells. These studies suggest that an important function of tuft cells involves production of immune-modulatory factors in response to injury and oncogenesis. Consistent with this, we show that pancreas-specific Pou2f3 ablation eliminates tuft cell formation and enhances disease progression. Collectively, these data demonstrate that tuft cells and, by inference, the associated metaplastic and neoplastic lesions, play a protective role early in pancreatic injury and tumorigenesis. Co-opting these mechanisms may provide new strategies for detection and early interveation of PDA.

A Multiscale Map of the Stem Cell State in Pancreatic Cancer

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Drug resistance and resultant relapse remain key challenges in pancreatic cancer, and are in part driven by the inherent heterogeneity of the tumor that prevents effective targeting of all malignant cells. To better understand the pathways that confer an aggressive phenotype and drug resistance, we utilized a combination of RNA-seq, ChIP-seq and genome-wide CRISPR screening to systematically map molecular dependencies of pancreatic cancer stem cells, highly drug resistant cells that are also enriched in the capacity to drive tumor progression. Integration of these data revealed an unexpected role for immuno-regulatory pathways in stem cell self-renewal and maintenance in autochthonous tumors. In particular, Retinoic acid receptor-related orphan receptor gamma (ROR gamma), a nuclear hormone receptor known for its role in T cell differentiation and circadian rhythm, emerged as a key regulator of stem cells. ROR gamma transcriptional levels increased during pancreatic cancer progression, and binding motif analysis revealed an enrichment of ROR gamma motifs in stem cell-associated super-enhancers. Functionally ROR gamma inhibition, whether achieved via genetic or pharmacologic approaches, led to a striking defect in pancreatic cancer growth *in vitro* and *in vivo*, and improved survival in genetically engineered models. Collectively, these data reveal an unexpected co-option of immuno-regulatory signals by pancreatic cancer stem cells, and suggest that therapeutics currently being used for autoimmune indications should be evaluated as a novel treatment strategy for pancreatic cancer patients.

Pancreatic Cancer Science and Medicine

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Pancreatic ductal adenocarcinoma (PDAC) is almost uniformly lethal, and surgical resection of localized tumors followed by adjuvant chemotherapy is currently the only curative regimen. Unfortunately, most patients are diagnosed with advanced and surgically unresectable PDAC, due to a lack of early detection methods. Furthermore, such patients oftentimes have a rapid disease course due to the ineffectiveness of therapies. We developed mouse and organoid models of PDAC to explore the biological aggressiveness of PDAC and to address these clinical challenges. Our initial studies with organoid models have helped identify viable therapeutic approaches with common chemotherapies and targeted agents. Additionally, biomarkers of pancreatic cancer have been developed using epitomic approaches. Our exploration of basic scientific aspects of PDAC have also been enabled with the organoid models. In particular, organoid co-cultures with fibroblast precursors have revealed several subtypes of cancer fibroblasts in PDAC, and follow-up studies have confirmed the existence of these subtypes and led to new functional insights. Neoplastic cell biology can also be explored using organoid models, and pathways impacting metabolism, mitochondrial biology, and metastasis have been identified and may be discussed.

Poster Abstracts

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Histidine Kinase and the Missing Phosphoproteome in Human

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Phosphorylation is an important post-translational modification leading cell signaling. In organisms, 4 types of phosphate-protein linkage (SONAtes) exist on 9 different phosphoresidues. Histidine phosphorylation is established for 50 years but is unexplored. The acid-lability of phosphohistidine (pHis) means that techniques applied to study phosphate esters (phospho-Ser/Thr/Tyr) must be modified for the phosphoramidate (phospho-His/Arg/Lys). Advances, including anti-pHis monoclonal antibodies and stable mimics of pHis, facilitate the study of phosphoramidate bonds. The use of chemically phosphorylated synthetic peptides, confirmed to contain pHis by peptide dot blot, TL chromatography and MS/MS analysis, allowed us to test a method for purifying some pHis-containing peptides of a human cancer cell line (HeLa). This purification is a strictly non-acidic method preserving phosphoramidate bonds. Analysis by LC-MS/MS reveals conventional and non-conventional phosphorylation sites allowing a wider coverage of potential SONAtes phosphorylation. Specifically, the known pPGAM H11 peptide was enriched from human cells and validated by mass spectrometry. It strongly suggests that a non-acidic enrichment method allows the detection of N-phosphate residues by mass spectrometry. On another aspect, several His kinases are defined in prokaryotes, especially those involved in two-component system (TCS). However, in higher eukaryotes, NME/NM23 which was first identified as a non-metastatic protein, is the only known protein–histidine kinase. It has been related to several cancer and was shown to be upregulated in some tumors. Collaborative works are ongoing to study the involvement of this kinase in neuroblastoma. This conserved His kinase autophosphorylates its active site His118 and recently, a first sequence-specific pNME1/2 (H118) polyclonal antibodies was developed. These new antibodies establish a precedent for generating other sequence-specific pHis antibodies.

Modulating Tumor Hypoxia for Anti-tumor Immunity

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While immunotherapy represents a remarkable option for cancer treatment, about 70% of treated individuals see their tumors progress. While tumor heterogeneity is an important rate limiting factor for a therapeutic response, hypoxia is a feature common to most solid tumors and is generally abundant in a tumor microenvironment. Anti-tumor immune cells, like their cancer cell counterpart, require enough nutrients and oxygen delivery to maintain their functionality. However, prior studies on hypoxia were mainly from *in vitro* experiments. To better understand how hypoxia can potentially regulate anti-tumor immunity *in vivo*, we utilized PHD2+/- mouse model whereby enhanced endothelial normalization in aberrant blood vessels leads to increased tumor oxygenation. By injecting well-defined mouse melanoma cell line called YUMMER into wild-type (WT) vs PHD2+/- mice, we evaluated how increased oxygenation can mediate anti-tumor immunity. We observed reduced tumor hypoxia and more tumor-infiltrating CD8+ and CD4+ T cells in PHD2+/- mice, accompanied by decreased infiltration of tumor-promoting myeloid cells. Furthermore, delayed tumor growth is seen in PHD2+/- mice. In addition, T cells in the more hypoxic tumors of wild-type mice exhibited significantly higher expression of inhibitory molecules characteristic of exhaustion and were less functional. Based on these findings, we administered anti-PD1 & anti-CTLA-4 combination therapy to PHD2+/- vs WT mice to see if oxygenation could affect response to immunotherapy. Tumor growth was significantly delayed in treated PHD2+/- mice as compared to their wild-type counterpart. Our studies therefore highlight that enhanced oxygenation could be an important modulator for an effective anti-tumor immune response.

Investigating the Role of Cryptochrome 2 in Human Cancer

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Through numerous epidemiological studies, The World Health Organization (WHO) deemed disruption of circadian rhythms a probable carcinogen. Circadian rhythms regulate many physiological and cellular processes, while maintaining a ~24hr period. The molecular mechanisms by which disrupted circadian rhythms promote tumorigenesis remain unclear. Recently, our lab found that Cryptochrome 2 (CRY2) acts as an adapter to recruit c-MYC to the E3 ligase complex SKP-CULLIN-FBXL3 (SCF^{FBXL3}) to promote degradation of c-MYC, a known proto-oncogene transcription factor. We identified five recurrent CRY2 mutations (CRY2 D347H, S436R, R460C, D467N and S532L) in a variety of human cancers from The Cancer Genome Atlas (TCGA) (referred to as CRY2 TCGA mutations). We hypothesized that these mutations may disrupt the interactions between CRY2, FBXL3, and c-MYC and thereby promote c-MYC accumulation. We performed several biochemical and functional assays in parallel to investigate the impact of these mutations on CRY2 activities. While three of the mutants tested reduce the association of CRY2 with FBXL3 as expected, we unexpectedly found that one of the CRY2 TCGA mutants abolishes interaction between CRY2, CLOCK, and BMAL1 without affecting its ability to interact with FBXL3 or c-MYC. Thus, this mutation prevents the normal function of CRY2 within the circadian clock, which could affect cellular transformation as well.

Unbiased Functional Identification and Therapeutic Targeting of T cell Neoantigens in a Spontaneous Murine Squamous Cell Carcinoma

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The comparative resistance of some cancers including head and neck squamous cell carcinoma (HNSCC) to checkpoint blockade is speculated to derive from the low frequency of expressed somatic mutations targeted by T cells as neoantigens (NeoAg). SCC VII, a spontaneously arising murine squamous carcinoma resembling human HNSCC in several key features, is likewise poorly immunogenic as irradiated tumor cells alone fail to induce protective immunity within syngeneic hosts. Justifying use of this model to identify NeoAgs, we confirm activated $CD4^+$ and $CD8^+$ T cells are detectable and essential for vaccine efficacy of SCC VII and polyI:C co-administration. Whole-exome sequencing tumor versus normal genome identified 39 nonsynonymous missense mutations that were synthesized into 81 representative 20-mers. NeoAg-specific $CD4^+$ T cell IFN-γ responses were found against mutations of *Pik3ca*, *Ctnnd1*, and *Otud5* while both $CD4^+$ and $CD8^+$ T cells produced IFN-γ when stimulated by a single *Cltc* mutation during *in vitro* recall assays. Prophylactic immunization with a mixture of all stimulatory peptides protected hosts from subsequent tumor challenge. However, these peptides were not therapeutically beneficial *in vivo* unless the *Cltc* NeoAg, eliciting both $CD4^+$ and $CD8^+$ T cell responses, was used as an immunotherapy alone. Anti-PD-1 combinatorial blockade resulted in synergistic tumor rejection via boosting *Cltc*-specific responses and increasing response diversification via epitope spreading. These data show that a functional NeoAg identification platform can be used to select immunotherapeutically relevant targets and filtration of neoepitopes that co-prime both $CD4^+$ and $CD8^+$ T cell responses is superior for practical intervention of poorly immunogenic tumors.

Glioblastoma Stem Cells Reprogram Circadian Regulators to Provide a Novel Therapeutic Approach

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Glioblastoma are highly lethal cancers, containing self-renewing, stem-like glioblastoma stem cells (GSCs). Here, we show that GSCs and differentiated glioblastoma cells (DGCs) displayed robust circadian rhythms as that in normal brain cells. However, the dependence of these cells on core circadian regulators is distinct. Disruption of core circadian regulators BMAL1 or CLOCK showed limited toxicity on DGCs, and normal brain cells. In contrast, GSCs were exquisitely sensitive to disruption of core clock regulators, which induced cell cycle arrest, apoptosis and reduced *in vivo* tumor growth. Associated with differences in active chromatin regions, BMAL1 preferentially bound at metabolic network genes in GSCs, compared to neural stem cells (NSCs). Targeting *BMAL1* or *CLOCK* in GSCs attenuated mitochondrial metabolic function and reduced expression of the tricarboxylic acid (TCA) cycle enzymes. Pharmacologic agonists of negative circadian regulators, the REV-ERBs and Cryptochromes, downregulated stem cell regulators and reduced GSC growth. Thus, GSCs coopt circadian molecular regulators to promote maintenance and tumor metabolism, offering selective dependency amenable to therapeutic targeting.

Characterizing the Role of TIE2 and TIE2 Neutralization in Cancer Cell Dormancy and Bone Metastases

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Almost all patients with advanced breast (BCa) or prostate cancer (PCa) have incurable bone metastases. Cancer cells disseminated to bones can remain dormant and resistant to treatments, and new therapies are needed to kill them. Dormancy mechanisms seem similar to the ones of hematopoietic stem cell (HSC). The receptor TIE2 appears to regulate the dormancy of HSC and BCa cells. Thus, we aim to address the role of TIE2 in dormancy and bone metastases.In multiple datasets of BCa patients, high levels of *Tie2* mRNA in the primary tumor correlate with increased survival and time to metastases. In 6 BCa and 4 PCa cell lines tested, there was no or very low expression of TIE2 protein or mRNA. Using transduction, we obtained $TIE2^+$ MDA-MB-231 (BCa) and PC-3 cells (PCa). Expression of TIE2 was then gradually lost in proliferating cells, despite puromycin selection. So we used a conditional expression system where doxycycline-induced TIE2 expression. The continuous presence of doxycycline also led to the disappearance of $TIE2^+$ cells. These results suggest that there is no expression of TIE2 in growing cancer cells, consistent with the observation in Oncomine that there is less *Tie2* in the primary tumor of BCa patients compared to normal tissue.To neutralize TIE2 with a shark antibody (vNAR) and reverse dormancy, we used a synthetic vNAR library to isolate clones recognizing TIE2. After on-cell ELISA, we selected a clone that binds to the TIE2 extracellular domain. In silico analysis found that this clone could bind to the ligand-binding site, the FN2 or FN1 domain of TIE2, which could prevent its function.Overall our results suggest that there is no expression of TIE2 on growing cancer cells and that low levels of *Tie2* are associated with a longer time to metastasis, consistent with a longer dormancy. Neutralization of TIE2 with a vNAR could then be used to reverse dormancy and increase the sensitivity of cancer cells to treatments for patients at risk of bone metastases.

Genetic Analysis Reveals AMPK is Required to Support Tumor Growth in Murine Kras-dependent Lung Cancer Models

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The AMP-activated protein Kinase (AMPK) is a conserved regulator of cellular energy homeostasis that governs glucose and lipid metabolism in all eukaryotes. Analysis of AMPK function in cancer has suggested a multifaceted role for AMPK, where it can either repress or promote tumor growth depending on the context. However, no studies to date have examined AMPK function in autochthonous genetic mouse models of epithelial cancer where metabolic stress is most likely to be present. Here we examine the role of AMPK in murine $Kras^{G12D}$ mediated non-small cell lung cancer (NSCLC), a cancer type in humans that harbors frequent loss of function mutations in the Liver Kinase B1 (LKB1/STK11) tumor suppressor - the predominant upstream activating kinase of AMPK and twelve AMPK related kinases. Unlike LKB1 deletion, deletion of AMPK and complete loss of AMPK signaling in $Kras^{G12D}$ lung tumors did not lead to any acceleration of lung tumor growth. Moreover, genetic deletion of AMPK in the more aggressive $Kras^{G12D}$ p53 floxed context led to a striking reduction in lung tumor size and overall tumor burden. Further dissection of the function of AMPK in $Kras^{G12D}$ lung tumors revealed a critical role for AMPK in regulation of the coordinated lysosomal expression and regulation (CLEAR) network, through the Tfe3 transcription factor, which was required to support the growth of NSCLC. These findings demonstrate a requirement for AMPK to support the growth of $Kras^{G12D}$ dependent lung cancer through the induction of lysosomes, highlighting a previously unrecognized metabolic liability of NSCLC.

Role of Transforming Growth Factor Beta in Neuroblastoma Bone Metastasis

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Neuroblastoma (NB) is the most common extracranial solid tumor among pediatric cancers. A unique feature of NB is the high metastatic rate, where bone is one of the main sites of metastasis. Patients with NB usually develop osteolytic lesions such as the ones that in patients with breast and prostate cancer. Tumor cells in bone promote bone resorption and the release of growth factors from the bone matrix such as the transforming growth factor beta (TGF-beta). TGF-beta levels are increased in the bone microenvironment altering bone remodeling, promoting tumor growth, and bone destruction at the metastatic site. TGF-beta plays a main role in the establishment and progression of bone metastases from breast and prostate cancer and melanoma. Here, we hypothesize that TGF-beta also promotes bone metastasis from neuroblastoma. To test that we performed cellular biology experiments and we are working on the establishment of an *in vivo* model of NB bone metastases.First, we analyzed the basal expression of TGF-beta receptors I, II y III in Neuro-2a and SK-N-AS cell lines (mouse and human NB cells lines, respectively) by qRT-PCR. Both cell lines express similar levels of mRNA of the TGF-&#946 receptors. Then, we treated the cells with TGF-beta (5 ng/ml) and evaluated the mRNA expression of TGF-beta responsive genes involved in bone metastasis. PMEPA1, PTHrP and CXCR4 (TGF-beta target genes) were slightly modulated by TGF-beta in NB cells compared to control prostate cancer cells. TGF-beta treatment did not have any effect on the proliferation and migration of NB cells, however treatment with the soluble portion of the TGF-beta receptor III (also known as soluble Betaglycan) significantly decreased their proliferation rate.Furthermore, we are currently working on the establishment of an *in vivo* model of NB bone metastasis. Four weeks old Balb/c and CD1 Nu/Nu female mice received an intracardiac or intratibial inoculation of mouse (Neuro-2a) and human (SK-N-AS) NB cells, respectively, to cause the development of bone metastasis. Further studies include the analysis by X-ray and histology of the bones of the experimental mice. Bone metastasis is a frequent complication of NB, our aim is to reach a better understanding of this devastating disease and test new treatments.

Betaglycan, a Potential TGFBeta Inhibitor for the Treatment of Bone Metastases

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Betaglycan (BG) is a proteoglycan coreceptor for the Transforming Growth Factor Beta, TGFβ a major stimulator of breast cancer bone metastases. Bone metastases are incurable, and improved therapies to treat or prevent bone metastases are needed. BG offers a novel approach to treating bone metastases by inhibiting TGFβ both Smad and non-Smad signaling pathways and for direct effects on the tumor on regulation of migration, proliferation, invasion and angiogenesis. Loss of BG during cancer progression and direct regulation on tumorigenesis support the role of BG as a tumor suppressor. BG serves as precursor for a soluble form of the receptor, soluble BG (sBG). sBG inhibits TGFβ signaling, regulates cell adhesion and migration and inhibits tumor progression. sBG recombinant has been used effectively in animal models of cancer and fibrosis. Effects of BG or sBG on bone or tumors in bone have not been reported. We generated stable cells lines using a lentivirus system to overexpress BG and sBG in MDA-MB-231 cells. We characterized the sBG levels by Western blot and real time PCR. The generated clones expressed significant levels of BG and sBG compared to controls. The effect on migration and proliferation in the presence and ausence of TGFbeta. Future studies will also test role for glycosaminoglycans (GAGs) in cell proliferation, invasion and metastasis *in vivo*. Our studies aim to indicate whether proteoglycan-derived drugs would be useful against metastases established in the skeleton.

Mertk Mediates Brain Endothelial Cell Migration Promoting Angiogenesis

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Like all nascent tissues, tumors are dependent upon angiogenesis for growth, however, current anti-angiogenic therapies have proven to have only modest benefit for many cancer patients. It is increasingly clear that essential, as-yet-unknown signaling pathways must regulate the differentiation and morphogenesis of vascular endothelial cells in the context of cancer. We have discovered a novel Mertk-driven pathway inducing migration of brain microvascular endothelial cells (BMECs) with the potential to modulate tumor angiogenesis. Mertk belongs to the TAM family of receptor tyrosine kinases with essential homeostatic functions in multiple tissues. We show that *Mertk*$^{-/-}$ BMECs display impaired motility in a wound healing assay, transwell migration assay and vascular tube formation assay, effects which can be mimicked by treating *WT* BMECs with Mertk-inhibitors. Despite *WT* BMECs actively producing the TAM-ligand Gas6, their migratory phenotype is enhanced by supplementation with exogenous Gas6. Using RNAseq, we show that more than 10 angiogenic proteins (among others EphB1, Meox2 and Dll4) are downregulated in *Mertk*$^{-/-}$ BMECs compared to *WT* BMECs, effects also seen in *WT* BMECs treated with Mertk-inhibitors. We further show that Mertk-activation by Gas6 induces Akt-phosphorylation at S473 in a PI3K-dependent manner. Phosphorylated Akt then directly phosphorylates the transcription factor Foxo1 at S256, inducing its release from the iNOS promoter and translocation to the cytoplasm. In *Mertk*$^{-/-}$ BMECs, an increased nuclear presence of Foxo1 leads to upregulation of iNOS expression and a high production of NO. This may contribute to the increased permeability of the blood-brain-barrier previously described in *Mertk*$^{-/-}$ mice. Importantly, inhibition of Akt inhibits the Gas6-induced cell migration in *WT* BMECs showing that the PI3K-Akt-Foxo1 pathway is also involved in regulating the migratory response of BMECs. Specific inhibition of Mertk at the stage of surgical tumor removal may prevent tumor re-establishment by preventing neovascularization of remaining cancer islets.

Towards Understanding Intratumoral Heterogeneity; Exploring "Phenotypic Mosaicism" In a Mouse Model of Glioma

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The lethality of glioblastoma multiforme (GBM) and the failure of treatment is largely attributable to the heterogeneous properties of this cancer. We aim specifically to characterize the driving event for GBMs to acquire a heterogeneous phenotype during progression. Central to achieving this goal is the ability to interrogate possible sources of heterogeneity that guide cellular states that are similar to those identified in human patients. We therefore employ a mouse model of glioma that recapitulates the pathophysiology and gene expression signatures of human GBM. While initiated with identical oncogenic drivers, this mouse model unexpectedly displays heterogeneous phenotypes between animals. Our preliminary data using time-series single-cell transcriptomics approach show that transformed cells, even without acquiring additional genetic alterations, switch cellular states at the beginning of tumor formation and one population commit themselves to expand to establish gliomas that are mainly comprised of one cell type. We further observe the strong association of microenvironmental cues with glioma cell populations and their roles in the state transitioning of glioma cells. Through the characterization of mechanisms that drive phenotypic mosaicism, we hope to uncover general principles that govern tumor progression and heterogeneity, and then ultimately provide novel therapeutic strategies to cure GBM.

The AMPK-related Kinases SIK1 and SIK3 Mediate Tumor Suppressive Effects of LKB1 in NSCLC

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Mutations in the LKB1 (STK11) tumor suppressor are the third most frequent genetic alteration in non-small-cell lung cancer (NSCLC). LKB1 encodes a serine/threonine kinase that directly phosphorylates and activates a family of 14 kinases related to AMP-activated protein kinase (AMPK). While many of these 14 AMPK-related (AMPKR) kinases are poorly understood, some play well-conserved roles in cell growth and metabolism though which AMPKR kinases are most critical to the potent tumor suppressive function of LKB1 in the lung remains obscure. Here we combine CRISPR analysis of the AMPKR family in NSCLC cell lines and mouse models, revealing a surprising critical role for the SIK subfamily. Conditional genetic loss of Sik1 revealedincreased tumor growth in mouse models of Kras-dependent lung cancer, which were further enhanced by loss of the related Sik3. Since most known substrates of the SIKs control transcription, we performed gene expression analysis on primary GEMM tumors, revealing upregulation of AP-1, EMT, and IL6-JAK-STAT pathways in common between Lkb1- and Sik1/3-deficient tumors. Together, these results open new avenues of therapeutic exploration aimed against pathways directly regulated by SIK1 and SIK3 in the large fraction of NSCLC bearing LKB1 mutations, an aggressive tumor type for which there are currently no robust therapeutic strategies.

Establishment of *Ex Vivo* and *In Vivo* Models for the Study of Bone Remodeling

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The skeletal system is a highly dynamic tissue thanks to a process called bone remodeling that occurs throughout our life. Bone remodeling allows the bone to maintain its integrity and fulfill its functions through balanced cycles of resorption and formation. Worldwide, an osteoporotic fracture occurs every 3 seconds, 1 in 3 women over the age of 50 will experience osteoporotic fractures, as will 1 in 5 men aged over 50. To understand the mechanisms involved in bone remodeling, improve actual therapies, and continue the search of new potential therapeutic agents, it is necessary to develop models that recreate the bone microenvironment. Therefore, we worked on the establishment of three models. 1) As an osteoclastogenesis model, the bone marrow cells from BALB/C mice were cultured in the presence of M-CSF and RANKL. Nine days after induction, we validated the model by performing TRAP staining to quantify osteoclasts and used qRT-PCR to evaluate the expression of osteoclastic genes. 2) Osteoblasts were differentiated using isolated cells from neonatal mouse calvarias. Mouse calvarias were digested using collagenase, the isolated cells (osteoblast precursors and primary osteoblasts) were cultured for 21 days in the presence of ascorbic acid and β-glycerophosphate. Alkaline Phosphatase and Alizarin Red S staining were performed to validate the model. 3) Osteoporosis was modeled *in vivo* using ovariectomy. Ovariectomy was performed on females C57BL/6 of 8 and 16 weeks, one month later the mice were euthanized, and the bones were collected to evaluate bone loss by histology, or to measure the expression of genes associated with bone remodeling (Opg, Alpl, Runx2, Rankl, Acp5, Ctsk, MMP13). We found the right conditions to successfully recreate the bone microenvironment in ex vivo and *in vivo* models. Our results showed that we can use the implemented models to study bone formation and bone resorption. Unbalanced bone remodeling represents a severe public health problem. Worldwide, more than 200 million patients are affected by osteoporosis; we can use these models to reach a better understanding of this disease and assess potential therapeutic agents.

Exploration of Mechanisms of Drug Resistance in Brain Tumors

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Treatment of malignant tumors of the brain, gliomas, has remained limited. The standard of care is surgical removal followed by radiation and chemotherapy with the alkylating agent temozolomide (TMZ). Treatment with TMZ is most effective in the highest grade of glioma, glioblastoma, patients that have methylation of the primary resistance gene O6-methylguanine DNA methyltransferase (MGMT). Outcomes of treatment with TMZ are much less clear for lower grade gliomas. Computational analysis of cancer genomics data sets indicates that the majority of glioblastoma patients have a passenger deletion of MGMT due to loss of heterozygosity of PTEN. Therefore, the treatment with TMZ is likely affected by both the methylation and copy number status of MGMT. We seek to build mouse models of gliomas that better recapitulate the major genetic subtypes of adult gliomas. By doing so, we will directly test the association of MGMT copy number along the contribution of other dna damage repair pathways to the resistance of treatment with TMZ.

Metabolic Adaptation of Tissue-resident Macrophages in Cancer

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Tissue resident immune cells play a critical role in maintaining homeostasis and orchestratinghost defense. Resident macrophages represent cell populations that are uniquely adapted totheir specific niche, integrating multiple complex environmental cues to generate a maximallybeneficial response for their client epithelia. We propose a model in which the deregulatedproliferation of malignant epithelia co-opts resident macrophage homeostatic networks tofavor tumorigenesis. Using a genetically inducible mouse model of lung adenocarcinoma weobserved a striking expansion in the resident cells of the alveolus, the alveolar macrophage(AM). By engaging AM lipid metabolism—a metabolic program known to skew macrophagestowards an M2 like state—AM homeostatic clearance of the lipid-rich alveolar surfactant aids inthe maintenance of airway tolerance, critically important in the lungs where there is a high costto inflammation. As tumors progressed, AMs up-regulated surface expression of markersassociated with alternatively activated macrophages, decreased their production ofinflammatory cytokines, while increasing their lipid uptake and storage. AMs had functionallyaltered metabolic states with tumor-associated AMs having significantly increased rates ofbasal respiration as well as mitochondrial uncoupling. Induction of the transcription factorPPARγ is necessary for AM maturation, integrating AM surfactant clearance and consequentlylipid catabolism with their tolerant immune state. Pharmacological inhibition of PPARγ delayedtumor progression, reduced AM recruitment, while restoring their inflammatory cytokineproduction. Our data suggests that rewiring of AM metabolism, specifically via antagonism of PPARγ, has the potential to be a novel therapeutic target in the treatment of lung cancer.

DNA Damage-induced Cell Death Relies on SLFN11-dependent Cleavage of Distinct Type II tRNAs

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Previously we showed that as an intrinsic immunity factor, human SLFN11 inhibits HIV viral proteins syntheses by modulating tRNAs abundances and exploiting the biased codon usage of the virus. Transcriptome analysis revealed a strong positive correlation between SLFN11 expression and the sensitivity of tumor cells to DNA damaging agents (DDAs). We hereby report that SLFN11 preferentially inhibits translation of genes involved in DNA damage response and repair, including ATR and ATM, upon DDAs treatment based on their distinct codon usages without disrupting early DNA damage response signaling. Type II tRNAs, which include all serine and leucine tRNAs, are cleaved in a SLFN11-dependent manner in response to DDAs. The mRNAs encoded by genes with high TTA (Leu) codon usage such as ATR display utmost susceptibility to translational suppression by SLFN11 due to the extreme low abundance of cognate tRNA-Leu-TAA. Specific attenuation of tRNA-Leu-TAA sufficed to ablate ATR protein expression and restore DDA sensitivity of SLFN11-deficient tumor cells. Our study uncovered a novel mechanism of DNA damage response regulation mediated by SLFN11-dependent tRNA cleavage and codon-specific translational inhibition. Further investigation will reveal the roles of SLFN11 in DNA damage-related tumorigenesis and cancer therapies as an immunity factor.

Combined p53 Activation and BET Inhibition Exerts Synergistic Lethality in Acute Myeloid Leukemia

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The overall leukemia incidence rate is increasing by almost 2% per year, driven primarily by acute myeloid leukemia (AML). In 2017 alone, an estimated 5,970 new AML cases were diagnosed, and the 5-year survival rate is 27% (American Cancer Society 2017 reports). p53 (encoded by the *TP53* gene) is a classic pro-apoptotic tumor suppressor. Most human cancers inactivate p53 or the p53 pathway. Surprisingly, over 90% of AML patients retain wild type p53. The mechanism by which most AML disable or bypass the tumor suppressor function of p53 is still not well understood. Brd4 is a member of the BET (bromodomain and extra-terminal domain) family that plays a key role in the development and maintenance of AML. Brd4 has been indicated as a regulator of p53 target gene transcription. We find that concurrent p53 activation (via MDM2 inhibition) and BET inhibition induce synthetic lethality and achieve remarkable efficacy in human AML cell lines, primary human blasts and in mouse models. Mechanistically, our data suggests that Brd4 represses p53-mediated transcription activation and apoptosis in AML. This implies a novel oncogenic function for Brd4 in AML, as a p53 antagonist. This work also underscores the utility of wild type p53 as a therapeutic target in AML, especially in combination with other drugs. We have performed screens for new drugs that synergize with MDM2 inhibitors to kill AML cells. Hits from these screens are currently being validated.

Bicompartmental Effects of the Histone Deacetylase Inhibitor Entinostat Provide Therapeutic Benefits in Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers with limited therapeutic options. The challenge for effective therapies is to eliminate malignant pancreatic cancer cells (PCCs) in a microenvironment dominated by a pronounced stromal desmoplastic response. Here we report that entinostat, a histone deacetylase (HDAC) inhibitor, is capable of modulating both the neoplastic epithelium and stromal fibroblasts and providing therapeutic benefits in PDAC. We demonstrate that, entinostat drives activated pancreatic stellate cells (PSCs) towards an inactivated state featuring reduced myofibroblast properties and halted cell cycle. Mechanistically, entinostat specifically antagonizes the transcriptional program and restricts chromatin opening in PSC activation. Importantly, we found that this reprogramming capacity of entinostat is conserved in human cancer-associated fibroblasts (CAFs), and entinostat-treated CAFs fail to elicit the appropriate responses to the cytokines in the PDAC microenvironment. Coincident with its effect on CAFs, we found that entinostat induces cytostasis in PCCs by downregulating genes important for cell cycle progression. Using PDAC mouse models, we demonstrate that entinostat treatment significantly prolongs survival, reduces tumor burden, and delays tumor progression from differentiated, less advanced to undifferentiated, aggressive tumors. Notably, entinostat treatment preserves the tumor-stroma balance, thereby avoiding the detrimental outcomes associated with stromal depletion. Finally, we found that entinostat induces a systematic downregulation of DNA repair pathway components in PCCs, potentially inducing vulnerabilities. Indeed, combining entinostat with the DNA damage-inducing agent cisplatin enhances the therapeutic outcomes. Overall, this work highlights a novel therapeutic approach for targeting PDAC based on the bicompartmental reprogramming induced by HDAC inhibition.

Changes to Calcium Signaling during CD8 T cell Exhaustion

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Cancer immunotherapies attempt to revive intratumoral cytotoxic T lymphocytes (CTLs) from dysfunctional, 'exhausted' states to stimulate anti-tumor T cell responses, but it remains poorly understood how T cell exhaustion originally develops. The hallmarks of exhaustion – reduction in CTL functions, accumulation of inhibitory receptors, gene expression changes, and severe mitochondrial dysfunction – appear before CTLs become fully committed to exhaustion (illuminating a therapeutic window of opportunity). But a fundamental question that remains unknown is: which CTL signaling pathways trigger these changes in CTLs? One hypothesis is that adaptive feedback changes to calcium (Ca^{2+}) signaling events are key components of this clock, underlying both the observed gene expression changes and mitochondrial dysfunction. Our preliminary data indicate that that basal cytosolic Ca2+ levels are highly elevated in a subset of tumor infiltrating CTLs, and that this elevated state correlates with high expression of T cell activation and exhaustion markers. Specifically, elevated cytosolic Ca^{2+} levels are associated with high levels of the early-appearing exhaustion markers CD38, CD39, and PD1, but do not strongly associate with the late-exhaustion marker TIM3. Together these data suggest that a high resting Ca^{2+} state may represent an intermediate in the differentiation towards T cell exhaustion. Future work will focus on better understanding the expression states that lead to and arise from the high Ca^{2+} state, and how these relate to regulation of mitochondrial function. Understanding the molecular mechanism of commitment to T cell exhaustion will allow for the design of therapies that prevent its progression, thus creating 'inexhaustible' T cells capable of a durable response in immunotherapy.

Autophagic cell death restricts chromosomal instability during replicative crisis

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Crisis is a senescence-independent mechanism and has evolved as final barrier against oncogenic transformation by eliminating pre-cancerous cells with disrupted cell cycle checkpoints. It culminates in extensive cell death, thereby functioning as potent tumor suppressor. Cells rarely evade elimination and evolve towards malignancy, yet the mechanisms underlying cell death in crisis are not defined. Here we reveal a dominant role of macroautophagy in the death of both fibroblasts and epithelial cells transitioning through crisis. Autophagy activation emerged critical for cell death in crisis as its suppression promoted crisis bypass and continued proliferation, while genome instability accumulated. We demonstrate that telomere dysfunction specifically triggers autophagy, implicating a telomere-driven autophagy pathway that is not induced by intrachromosomal breaks. We show that telomeric DNA damage generates micronuclei with fragile nuclear envelopes that undergo spontaneous disruption. The resulting cytosolic chromatin fragments activate the cGAS-STING signaling pathway and engage the autophagy machinery to degrade vital cellular components. Our data point to autophagy as an integral component of the tumor suppressive crisis mechanism and suggest that loss of autophagy function is required for cancer initiation.

Key Mediator(s) of Paracrine Crosstalk within the Pancreatic Tumor Microenvironment: LIF or IL6?

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Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer death in the U.S. with the highest lethality and a median survival of less than 6 months. A prominent feature accounting for its dismal prognosis is the formation of dense stroma, providing a unique microenvironment for the intricate interaction between cancer and stromal cells. The stroma is pivotal for pancreatic cancer progression and drug resistance, not only just acting as a mechanical barrier to impede drug access, but also providing a unique growth permissive environment, favoring sustained activation of stromal cells, promoting tumor progression and metastasis, and helping tumor cells circumvent immune surveillance. The pancreatic stellate cell (PSC) has recently been identified and recognized as a key player in the formation and turnover of pancreatic tumor stroma. It has been proposed that the reciprocal communication between pancreatic cancer cells (PCCs) and PSCs results in the formation of a vicious cycle, comprising not only the sustained activation of PSCs but also the enhanced tumor progression and metastasis. Given the critical roles of this cell-cell crosstalk, we postulate that a better understanding of it can shed light on the vulnerability amenable for therapeutic target. To this end, using systematic MS-based integrated functional screen, we investigated the dynamic changes in both the composition and cell signaling capacity of the secretomes of pancreatic stellate cells (PSCs) and pancreatic cancer cells (PCCs), and found that, among many intriguing findings, the IL6 family cytokines, mainly secreted by activated stromal PSCs, play important roles in this intercellular communication. Strikingly, in contrast to the widespread belief that IL6 is involved in PDAC, our in-depth side-by-side comparison of IL6 and leukemia inhibitory factor (LIF) in mouse and human PDAC underscored the importance of LIF versus IL6 in human PDAC. These new findings provide a valuable resource to the field for ascertaining differences between the mouse model and the human disease.

Dismissal of RNA Polymerase II Underlies a Large Ligand-Induced Enhancer Decommissioning Program

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Nuclear receptors induce both transcriptional activation and repression programs responsible for development, homeostasis, and disease. Here, we report a previously overlooked enhancer decommissioning strategy underlying a large estrogen receptor alpha (ERα)-dependent transcriptional repression program. The unexpected signature for this estrogen-induced program resides in indirect recruitment of ERα to a large cohort of pioneer factor basally active FOXA1-bound enhancers that lack cognate ERα DNA-binding elements. Surprisingly, these basally active estrogen-repressed (BAER) enhancers are decommissioned by ERα-dependent recruitment of the histone demethylase KDM2A, functioning independently of its demethylase activity. Rather, KDM2A tethers the E3 ubiquitin-protein ligase NEDD4 to ubiquitylate/dismiss Pol II to abrogate eRNA transcription, with consequent target gene downregulation. Thus, our data reveal that Pol II ubiquitylation/dismissal may serve as a potentially broad strategy utilized by indirectly bound nuclear receptors to abrogate large programs of pioneer factor-mediated, eRNA-producing enhancers.

Atropisomeric Pyrrolopyrimidine Ret Kinase Inhibitors

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The active sites of oncogenic kinases are highly conserved throughout the kinome, making it difficult to selectively inhibit a specific kinase leading to unwanted off-target effects. 80% of FDA approved kinase inhibitors contain at least one rotational axis between two aromatic rings. This leads to an extended form of chirality called atropisomerism, where the two different rotational conformers can either exist as a rapidly racemizing mixture or isolable enantiomers. Most bioactives, as designed, exist as a rapidly interconverting atropisomeric mixture, however, when they bind to their target active site, they tend to do so in an atropisomeric fashion. The presence of the non-relevant atropisomer via interconversion or stable racemic mixture can result in off-target inhibition. Our lab exploits atropisomerism as a selectivity filter to represent a general strategy to increase kinase selectivity. In our original report, we rigidified a rapidly interconverting promiscuous kinase inhibitor by adding steric bulk adjacent to the axis of chirality and found the (R_a)-atropisomer to be a mildly potent RET inhibitor (IC_{50} 1857 nM) and be 7x more selective than the parent interconverting inhibitor. Though this data represents a proof of concept, the inhibitors must be optimized for increased potency and selectivity to fully exploit this strategy. To accomplish this, we first identified potential analogs through *in silico* screening of various substituent combinations of the (R_a)-atropisomer. We synthesized the top scoring molecules and evaluated them in biochemical and cellular assays. Through successive optimizations, our lead compound is a potent RET inhibitor (K_i 0.9 nM) containing 175x selectivity over SRC and 700x selectivity over VEGFR2. As more compounds are tested, we will iteratively optimize the RET inhibitors potentially leading to valuable chemical probes while demonstrating that leveraging atropisomerism is a viable selectivity filter.

Using Mathematical Modeling to Understand Oncogenic G-protein-coupled Receptor Signaling

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Certain oncogenic mutations are known to be drivers of a variety of cancers. These mutations create proteins with different biochemical properties than their wild-type (WT) counterparts. The proteins encoded by oncogenes are involved in complex intracellular signaling networks. The interactions between these networks and the oncogene determine the effect of a given mutation. This can be difficult to infer by simply knowing the protein interactions involved. To elucidate the systems level consequences of oncogenic mutations, mathematical modeling has emerged as a powerful tool. Here we present our current progress on the development of a mechanistic mathematical model for oncogenic G-protein-coupled receptor (GPCR) signaling. GPCRs are the largest family of membrane-bound receptors and are the target of approximately 30% of all clinically available drugs. GPCRs mediate a broad range of extracellular signals including those by hormones, neurotransmitters and other small-molecules. It has recently come to light, through modern deep-sequencing data, that GPCRs and associated G proteins can play a significant role in many cancers. Here we present a mechanistic model for the single most frequent oncogenic mutation found in G proteins, the R201C mutation in GNAS (which encodes for the $G\alpha_s$ subunit), which is common in pancreatic, colorectal and bone cancer, among others. Recent biochemical characterization of this mutation has raised interesting questions about its potential role in tumorigenesis. Our modeling results help to elucidate the potential role of this mutation in cancer. Specifically, we quantify the activation of the main effector of $G\alpha_s$ (adenylyl cyclase) by WT and mutant $G\alpha_s$GTP and $G\alpha_s$GDP. We also present our preliminary work on the development of a model for GNAQ/GNA11 mutations, which are found in over 90% of uveal melanomas.

Loss of the SWI/SNF Complex Tumor Suppressor ARID1A Results in cGAS-dependent Activation of Interferon Stimulated Genes

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ARID1A is a dedicated subunit of the SWI/SNF chromatin remodeling complex and has been shown to be genetically inactivated in several types of cancers, suggesting that it has a tumor suppressor role. In this study, we found that loss of ARID1A results in the induction of interferon-stimulated genes, specifically those associated with the interferon-related DNA damage transcription signature (IRDS). This is not due to the direct repression of IRDS genes by ARID1A, but rather to cytosolic recognition of double stranded DNA (dsDNA) present in micronuclei. Indeed, we show that micronuclei are markedly increased following conditional deletion of ARID1A in mouse embryonic fibroblasts (MEFs). Moreover, we detected a delay in the G2/M stage of the cell cycle in ARID1A-deficient MEFs. We also observed increased micronuclei incidence in two colorectal cancer cell lines engineered with ARID1A mutations. These micronuclei were bound by Lamin B1 and stained positive for dsDNA, gH2AX and the cytosolic DNA sensor cGAS. Further, ISG induction was dependent on the cGAS/STING/TBK1 pathway. These data suggest that loss of ARID1A results in cell cycle delay following dsDNA breaks, leading to the formation of micronuclei and activation of cGAS/STING-dependent antiviral innate immune response. Finally, using available genomic data from the TCGA database, we found a significant upregulation of IRDS genes in ARID1A mutant colon and gastric cancers, compared to those with intact ARID1A, highlighting the relevance of our study. Our findings offer insight into how ARID1A loss contributes to processes that activate the immune response in cancer cells, which has implications for the tumor-immune microenvironment and immune checkpoint blockade therapy of ARID1A mutant cancers.

Innate Immune Sensing of mtDNA Instability Protects the Nuclear Genome

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The mammalian genome comprises nuclear DNA (nDNA) derived from both parents and mitochondrial DNA (mtDNA) that is maternally inherited and encodes essential proteins required for oxidative phosphorylation. Thousands of copies of the circular mtDNA are present in most cell types and packaged by TFAM into higher-order structures called nucleoids. Mitochondria are platforms for antiviral signaling and, due to their bacterial origin, mtDNA and other mitochondrial components trigger innate immune responses and inflammatory pathology. We showed previously that mtDNA instability in *Tfam*$^{+/-}$ mice activates the cGAS-STING-TBK1 pathway resulting in interferon stimulated gene (ISG) expression that primes anti-viral immunity. Here we demonstrate that this form of mtDNA stress does not basally activate NF-κB signaling or interferon gene expression typical of an antiviral response. Instead, a specific subset of ISGs is activated by the unphosphorylated form of ISGF3 (U-ISGF3) that provides resistance to DNA damaging agents via an enhanced rate of nDNA repair. The chemotherapeutic doxorubicin also results in expression of ISGs that is dependent on mtDNA and cGAS-STING-TBK1 activation. Finally, global analysis of cancer cell lines indicates an inverse correlation between Tfam expression and that of ISGs. Therefore, we propose that mtDNA is a sensor of genotoxic stress that signals the need for nDNA repair, but that chronic activation of this mtDNA-stress pathway contributes to resistance to cancer therapies.