FOX CHASE CANCER CENTER
21st ANNUAL TRAINEE RESEARCH CONFERENCE

SCHEDULE

8:30 - 9:00 **CONTINENTAL BREAKFAST** **FCCC Auditorium Lobby**

9:00 - 9:05 **Welcome** **FCCC Auditorium**
J. Robert Beck, M.D., Ph.D.
Chief Academic and Administrative Officer

9:05 - 10:30 **ORAL PRESENTATIONS I** **FCCC Auditorium**

Emily A. Arturo: "Mammalian Phenylalanine Hydroxylase Assembles to Two Architecturally Distinct Tetramers"
Jimson D'Souza: "Identification of Kinome Signatures in Genetically Defined Subgroups of Gastrointestinal Stromal Tumor"
Anna Nikonova: "Repurposing of Targeted Cancer Therapies for Autosomal Dominant Polycystic Kidney Disease (ADPKD)"
Yifan Wang: "BRCA1 N-Terminal Deficient Protein Promotes PARP Inhibitor and Platinum Resistance"
Pengtao Jiang: "The Candidate Breast Cancer Gene CCDC170 Regulates Golgi- Derived Microtubule (MT) Dynamics"

10:30 - 11:30 **COFFEE BREAK & POSTER SESSION I** **2nd Floor Mezzanine Women's Center**

11:30 - 12:30 **KEYNOTE PRESENTATION** **FCCC Auditorium**
Dr. Rugang Zhang, Wistar Institute, Philadelphia
"Metabolism, Chromatin Structure and Senescence Phenotypes"

12:30 - 1:30 **LUNCH**

1:30 - 3:00 **ORAL PRESENTATIONS II** **FCCC Auditorium**

Oxana Dmitrieva: "The Role of IL-1R-Signaling in Tumor Elicited Inflammation in Colon Cancer"
Rossella Tricarico: "TET-TDG Inactivation Enhances Intestinal Tumorigenesis by Modifying the Epigenome in the APC^{MIN} Mouse Model"
Shengliang Zhang: "Reactive Oxygen Species and ERK2 Phosphorylation are required for NSC59984 to Induce Mutant P53 Protein Degradation and Restore P53 Signaling"
Bryan Harris: "Reduced RPL22 Expression is seen in MDS/AML and Predisposes Hematopoietic Cells to Leukemogenesis"
Daniela Di Marcantonio: "PKCε Regulates Redox Biology to Support Acute Myeloid Leukemia Survival"

3:00 - 4:00 **POSTER SESSION II** **2nd Floor Mezzanine Women's Center**

3:00 - 5:00 **EDWARD DAVID LUSTBADER RECEPTION** **2nd Floor Mezzanine Women's Center**

5:00 **BARUCH AND JEAN BLUMBERG AWARDS CEREMONY**

Edward David Lustbader

Edward David Lustbader was a biostatistician at Fox Chase Cancer Center where he was a member of the Nobel Prize winning research program that identified the hepatitis B virus and its role in liver cancer. A researcher of international stature in biostatistics and genetic epidemiology, Dr. Lustbader's work focused on the development and application of statistical methods used to understand the causes of cancer. Dr. Lustbader joined the Fox Chase Cancer Center in 1972, where he later became a Member in the Division of Population Science. He worked closely with Dr. Baruch Blumberg in identifying individuals at risk of developing the hepatitis B virus infection and, subsequently, primary liver cancer. Dr. Lustbader also made seminal contributions to the understanding of the relationship between diet and cancer. Working with geneticist Alfred G. Knudson Jr., M.D., Ph.D., Dr. Lustbader developed and applied statistical methods to determine the genetic basis of cancer.

Born in Baltimore, Maryland, Dr. Lustbader spent most of his professional life working in the Philadelphia area. After graduating from the Case Institute of Technology (now Case-Western Reserve University) in 1967, he came to the Delaware Valley to work for General Electric Company as a statistician and began graduate studies at the University of Pennsylvania. He received his M.S. degree from the Wharton School and his Ph.D. in statistics from the University in 1972. He was the author or co-author of more than 100 scientific publications.

The faculty, staff, and postdoctoral community at the Fox Chase Cancer Center gratefully acknowledge the Friends and Family of Dr. Lustbader for their continued support of the Annual Postdoctoral Research Conference.

Rugang Zhang, PhD is a Professor and Co-Leader of the Gene Expression and Regulation Program at the Wistar Institute, Philadelphia; as well as an adjunct faculty member of the Department of Genetics at the University of Pennsylvania. Dr. Zhang carried out postdoctoral studies at Fox Chase Cancer Center where he excelled and published key articles describing the relationship between heterochromatin and cellular senescence. He was subsequently promoted to Assistant Professor, and soon after initiating his independent laboratory he established himself as a leader in the cellular senescence field. Dr. Zhang moved his laboratory to the Wistar Institute in 2012 and continues to study basic mechanistic insights into the regulation of cellular senescence and mechanism-guided ovarian cancer therapy research. He obtained his PhD from the Shanghai Institute of Biochemistry and Cell Biology of Chinese Academy of Sciences, China. He is also a graduating member of the Department of Defense Ovarian Cancer Academy and has co-authored more than 60 peer-reviewed publications.

ORAL PRESENTATIONS SESSION I

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MAMMALIAN PHENYLALANINE HYDROXYLASE ASSEMBLES TO TWO ARCHITECTURALLY DISTINCT TETRAMERS

Emilia (Emily) C. Arturo[1,2], Kushol Gupta[3], Annie Heroux[4], Linda Stith[1], Penelope J. Cross[5,6,7], Emily J. Parker[5,6], Patrick J. Loll[2], and Eileen K. Jaffe[1]

[1]Fox Chase Cancer Center, Philadelphia, PA; [2]Drexel University College of Medicine, Philadelphia, PA; [3]University of Pennsylvania, Philadelphia, PA; [4]Brookhaven National Laboratory, Upton, NY; [5]University of Canterbury, New Zealand; [6]University of Auckland, New Zealand

Improved understanding of the relationship among structure, dynamics, and function for the enzyme phenylalanine hydroxylase (PAH) can lead to needed new therapies for phenylketonuria, the most common inborn error of amino acid metabolism. PAH is a multi-domain homo-multimeric protein whose conformation and multimerization properties respond to allosteric activation by the substrate phenylalanine (Phe); the allosteric regulation is necessary to maintain phenylalanine below neurotoxic levels. A recently introduced model for allosteric regulation of PAH involves major domain motions and architecturally distinct PAH tetramers (Jaffe, E.K., *et al*., 2013, *Arch Biochem Biophys,* **530**:73-82). Herein we present the first X-ray crystal structure for a full-length mammalian (rat) PAH in an auto-inhibited conformation. Chromatographic isolation of a monodisperse tetrameric PAH, in the absence of Phe, facilitated determination of the 2.9 Å crystal structure. The new structure supersedes a composite homology model that had been used extensively to rationalize phenylketonuria genotype-phenotype relationships. Small-angle X-ray scattering (SAXS) confirms that this tetramer, which dominates in the absence of Phe, is different from a Phe-stabilized allosterically activated PAH tetramer. The lack of structural detail for activated PAH remains a barrier to complete understanding of phenylketonuria genotype-phenotype relationships. Towards this end, model building in concert with SAXS analysis is used to test possible conformations of the activated PAH tetramer. The use of SAXS and X-ray crystallography together to inspect PAH structure provides the most complete view of the enzyme that was not possible with prior partial crystal structures, and facilitates interpretation of a wealth of biochemical and structural data that was hitherto impossible to evaluate.

IDENTIFICATION OF KINOME SIGNATURES IN GENETICALLY DEFINED SUBGROUPS OF GASTROINTESTINAL STROMAL TUMOR

Jimson W. D'Souza, Katherine Johnson, Martin Belinsky, Margaret von Mehren, James Duncan, and Lori Rink

Fox Chase Cancer Center, Philadelphia, PA

Management of gastrointestinal stromal tumor (GIST) has been revolutionized by the identification of activating mutations in the receptor tyrosine kinases (RTK), KIT and PDGFRA, and the clinical application of imatinib mesylate (IM) and other RTK inhibitors (sunitinib & regorafenib) in the advanced disease setting. However, clinical resistance to IM remains a challenge. Limited options exist for patients (~20%) that are refractory at the start of treatment and 50% of patients in the advanced setting experience disease progression after two years. GIST can be divided into three major genotypic subtypes: *KIT* mutants (75-80%), *PDGFRA* mutants (5-7%) and *KIT*/PDGFRA wild type (WT) GIST (10-15%), each of which has distinctive global gene expression and genomic profiles, clinical and pathological features and varying response profiles to RTK inhibitors. Importantly, global kinome profiles for these GIST subtypes are as yet undefined. Historically, the majority of unresectable and metastatic GISTs have been treated with the sequential application of the inhibitors IM, sunitinib, and regorafenib, regardless of genotype. The aim of this study was to identify novel targets within the subsets described above that may lead to a paradigm shift where first-line therapies can be tailored to tumor genotypic status in the clinical setting. We utilized a novel proteomics approach combining protein kinase-capture beads and mass spectrometry to explore simultaneously the majority of the active kinome in IM-naïve GIST patient specimens. This unbiased quantitative approach provides the 'big picture' of tumor kinase activity. To date, using this technology we have defined the basal activation state of the kinome in these molecularly and clinically distinct GIST subtypes. Current efforts are focused on validating the kinases and pathways identified in these studies in an independent GIST sample set and further evaluating them as potential targets in a panel of GIST cell lines.

REPURPOSING OF TARGETED CANCER THERAPIES FOR AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD)

Anna Nikonova, Alexander Deneka, Vladislav Korobeynikov, Harvey Hensley, Brian Egleston, and Erica Golemis

Fox Chase Cancer Center, Philadelphia, PA

Autosomal dominant polycystic kidney disease (ADPKD) is an inherited progressive genetic disease that affects 1 in 500 people. In this untreatable syndrome, normal kidney tissue is gradually replaced with fluid-filled cysts resulting in end-stage renal disease for most patients. In ADPKD, inherited mutations in polycystins 1 or 2 (encoded by *PKD1* and *PKD2*), which function as a heterodimeric signaling hub at the cell cilium, abnormally activates signaling pathways regulating cell proliferation, migration, and response to environmental cues. Intriguingly, studies of the signaling defects associated with ADPKD have increasingly identified parallel defects to those seen in cancer, in spite of the very different presentation of ADPKD and solid tumors.

We have been evaluating targeted signaling inhibitors in ADPKD models to probe the biological differences between this disease and cancer, and to determine whether advanced cancer drugs are effective in ADPKD. We will explore the signaling interactions between PKD1 mutations, control of ciliary dynamics, and clinical agents with potential efficacy in ADPKD, with a goal of optimizing therapeutic options for patients with ADPKD. In published and ongoing work, we have found that HSP90 inhibition in *Pkd1*$^{-/-}$ mice reduces initial renal cyst formation and slows the progression of these phenotypes in mice with pre-existing cysts. Treatment of kidney epithelial cells with HSP90 inhibitors (ganetespib and STA-2842) leads to the fast resorption of primary cilia. We now have an approval from our collaborators at Synta Pharmaceuticals to use ganetespib in *Pkd1*$^{-/-}$ mouse model. We have been using ganetespib in extended dosing experiments, finding the drug well-tolerated out to 13.5 months to date. These results provide very strong support for the idea that HSP90 inhibition represents a novel and valuable therapeutic concept for ADPKD.

BRCA1 N-TERMINAL DEFICIENT PROTEIN PROMOTES PARP INHIBITOR AND PLATINUM RESISTANCE

Yifan Wang, John J. Krais, Andrea J. Bernhardy, Emmanuelle Nicolas, Kathy Q. Cai, Maria I. Harrell, Hyoung H. Kim, Erin George, Elizabeth M. Swisher, Fiona Simpkins, and Neil Johnson

Fox Chase Cancer Center, Philadelphia, PA

Introduction: Tumors harboring *BRCA1* mutations initially respond well to platinum and PARP inhibitor therapy; however, resistance invariably arises and is a major clinical problem. The *BRCA1*^{185delAG} allele is a common founder mutation located close to the protein translation start site, thought to produce a short peptide devoid of function.

Experimental procedures: In this study, we utilized the SUM1315MO2 breast cancer cell line that harbors a *BRCA1*^{185delAG} mutation to study mechanisms of PARP inhibitor and platinum resistance. SUM1315MO2 cells were cultured in the presence of increasing concentrations of the PARP inhibitor rucaparib or cisplatin until rucaparib resistant (RR) and cisplatin resistant (CR) clones emerged.

Results: DNA sequencing revealed that no *BRCA1* gene reversion mutations were present in resistant cells. Western blotting showed that BRCA1 protein was undetectable in both SUM1315MO2 parental and resistant clones using the N-terminal specific antibody. However, the C-terminal specific antibody identified a more quickly migrating band, compared to wild-type BRCA1 protein. The abundance of N-terminal deficient BRCA1 protein was low in parental cells, but the expression was elevated in both RR and CR clones. The peptide sequencing suggested that translation initiation occurred downstream of the frameshift mutation, likely at the Met-297 codon in SUM1315MO2 cells. Therefore, this protein was devoid of the extreme N-terminal RING domain-containing region that mediates interaction with BARD1. To investigate the functionality of the N-terminal deficient BRCA1 protein, we measured BRCA1 and RAD51 irradiation-induced focus formation by immunofluorescence. Both RR and CR clones demonstrated an increase in the number of cells with BRCA1 foci or RAD51 foci compared to parental cells. Additionally, BRCA1 siRNA treated RR or CR cells were more sensitive to rucaparib or cisplatin compared to scrambled siRNA-treated control cells. Ectopic overexpression of Met-297 BRCA1 promoted partial PARPi and cisplatin resistance *in vitro* and *in vivo*. Furthermore, N-terminal deficient BRCA1 protein expression was detectable in recurrent carcinomas from germline *BRCA1*^{185delAG} mutation carriers.

Conclusions: Taken together, these results provide evidence for a novel, mutation location-dependent mechanism of PARP inhibitor and platinum resistance.

THE CANDIDATE BREAST CANCER GENE *CCDC170* REGULATES GOLGI-DERIVED MICROTUBULE (MT) DYNAMICS

Pengtao Jiang, Yueran Li, Andrey Poleshko, Valentina Medvedeva, Yan Zhou, Carolyn M. Slater, Trinity Pellegrin, Richard A. Katz, and Xiaowei Chen

Fox Chase Cancer Center, Philadelphia, PA

Numerous genome-wide association (GWA) and subsequent validation studies (>50) have linked the *C6ORF97*/*CCDC170-ESR1* locus (6q25.1) to a risk for breast cancer (BCa). Our previous differential allele-specific expression (DASE) studies identified *CCDC170* specifically as a candidate breast cancer susceptibility gene. However, there have been no functional studies of the wild type CCDC170 protein. Here we report for the first time that the wild type CCDC170 protein localizes to the Golgi apparatus, and BCa-associated truncations of CCDC170 result in loss of Golgi localization. Overexpression of CCDC170 triggers Golgi reorganization and enhances Golgi-derived MT stabilization and MT acetylation driven by the acetyltransferase ATAT1. Golgi-derived MTs regulate cellular polarity and motility, and overexpression of CCDC170 inhibits cell movement, while CRISPR knockout of the *CCDC170* gene enhances cell migration. Lastly, we identified the candidate CCDC170 functional binding partners that are consistent with its localization and proposed function. These partners may serve to mediate the acetylation and stabilization of MTs. Taken together, our findings demonstrate that CCDC170 plays an essential role in Golgi MT organization and stabilization, and provides a mechanism for how perturbations in CCDC170 could alter cell polarity, and thereby drive BCa and other abnormalities.

ORAL PRESENTATIONS SESSION II

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Oxana Dmitrieva: "The Role of IL-1R-Signaling in Tumor Elicited Inflammation in Colon Cancer"

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THE ROLE OF IL-1R-SIGNALING IN TUMOR ELICITED INFLAMMATION IN COLON CANCER

Oxana Dmitrieva[1,2], Vivian Hou[1], David Posocco[1], and Sergei Grivennikov[1]

[1]Fox Chase Cancer Center, Philadelphia, PA; [2]NRC Institute of Immunology FMBA, Moscow, Russia

Many solid tumors are characterized by the presence of immune infiltrates, which are able to enhance the expression of inflammatory mediators as well as to further recruit inflammatory cells to the local microenvironment - a process referred to as tumor elicited inflammation (TEI). In colorectal cancer, specifically, early oncogenic events lead to deterioration of the integrity of epithelial barriers, allowing translocation of microbial products and induction of pro-inflammatory cytokine expression. It has been previously demonstrated that increased levels of RORγt and IL17 correlate with poor prognosis in stage II CRC – implying a possible mechanistic role of TEI in CRC progression. The intricate mechanistic details of TEI remains to be worked out, however we and others showed that microbial-driven expression of IL23 stimulates production of its downstream pro-inflammatory and pro-tumorigenic effector cytokines (e.g. IL-17A, IL-17F and IL-22). We hypothesized that members of IL-1 family (IL1α, β) may play a distinct role in the induction of tumor elicited inflammation and CRC progression, acting in parallel with the established IL23 –dependent pathway of IL-17A induction to exacerbate CRC tumorigenesis. We found that IL1α and IL1β are overexpressed by CRC tumors. Using conditional IL-1R knockout mice, we demonstrated that IL-1R is expressed on epithelial, lymphoid and myeloid cells, and that IL-1 is able to signal through innate lymphoid cells (ILC's), CD4-Tcells, and TCRγδ cells in CRC tumors, resulting in enhanced pro-tumorigenic IL-17 production. Pharmacological inhibition of IL-1 reduced IL-17 production while IL-1R ablation in vivo impacted CRC tumorigenesis. In addition, we revealed an important role of IL-1R signaling on myeloid cell population in CRC tumors. Moreover conditional knockout of IL1R on CD11b+ cells showed completely opposite phenotype from IL1R-ko on T-cells. Altogether, IL-1/IL-1R signaling pathway is an important regulator of Tumor elicited inflammation and immunity in CRC tumors. thereby showing a significant impact on tumor growth.

TET-TDG INACTIVATION ENHANCES INTESTINAL TUMORIGENESIS BY MODIFYING THE EPIGENOME IN THE *APC*^{MIN} MOUSE MODEL

Rossella Tricarico[1], Jaroslav Jelinek[2], Gabrielle Scher[1], Harry Cooper[1], Wen Chi Chang[1], Margie Clapper[1], Yan Zhou[1], Karthik Devarajan[1], Jean Pierre Issa[2], and Alfonso Bellacosa[1]

[1]Fox Chase Cancer Center, Philadelphia, PA; [2]Temple University, Philadelphia, PA

Genetic and epigenetic events contribute to colorectal cancer (CRC), the second leading cause of cancer-related deaths in Western countries. Currently, there is an increasing emphasis to investigate epigenomic alterations in CRC to improve prevention, diagnosis and prognosis of this disease. Ten-Eleven Translocation (TET) family dioxygenases play a crucial role in active DNA demethylation by oxidizing 5-methylcytosine to 5-hydroxymethylcytosine (5hmC) and converting 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). The DNA repair enzyme Thymine DNA Glycosylase (TDG) is also involved in DNA demethylation downstream of the TETs by removing 5fC and 5caC. *TET1* and *TDG* sequence changes have been identified in intestinal tumors by next generation sequencing. Moreover, loss of 5hmC and gain of 5caC, biomarkers of TETs and TDG defect, respectively, have been found in CRC tumors by us and other groups.

To investigate the biological significance of the TET-TDG demethylation axis in CRC, we crossed *Tet1* and *Tdg* mutant mice with *Apc*^{Min} mice, a model of intestinal tumorigenesis. We discovered that *Tdg* and/or *Tet1* inactivation enhances tumorigenesis by increasing the number, size, and invasive features of colonic adenomas. In comparison to control *Apc*^{Min} mice, methylome analysis revealed progressive loss of global DNA hypomethylation in colonic adenomas from *Tet1* and *Tdg*-deficient mice, and hypermethylation of CpG islands in *Tet1*-deficient mice. The impact of these methylation changes on gene expression is being evaluated by RNA sequencing of colonic adenomas. Taken together, these findings demonstrate the important role of active DNA demethylation mediated by TET-TDG in reducing intestinal tumor formation, by modulating the epigenome. This study expands our understanding of intestinal tumorigenesis and suggests a novel mechanism of epigenetic deregulation with diagnostic, therapeutic and prognostic implications.

REACTIVE OXYGEN SPECIES AND ERK2 PHOSPHORYLATION ARE REQUIRED FOR NSC59984 TO INDUCE MUTANT P53 PROTEIN DEGRADATION AND RESTORE P53 SIGNALING

Shengliang Zhang, Lanlan Zhou, David T. Dicker, and Wafik S. El-Deiry

Fox Chase Cancer Center, Philadelphia, PA

Tumor suppressor p53 is mutated in over 50% of human cancers. p53 mutation abolishes wild-type p53 function and also endows mutant p53 with a gain-of-function (GOF) that drives tumor growth and drug resistance. Targeting mutant p53 is an attractive strategy for cancer therapy. We recently reported (Zhang et al., Cancer Research, 2015) a small-molecule NSC59984 with dual capabilities to restore p53 signaling and destabilize mutant p53 protein (depleting GOF). We now demonstrate the role of reactive oxygen species (ROS) and ERK2 in the mechanism of action of NSC59984. We observe a sustained-phosphorylation of ERK2 in cancer cells treated with NSC59984. Blockade of ERK2 rescues mutant p53 from NSC59984-mediated degradation, and inhibits restoration of p53 signaling in mutant p53-expressing cells. Thus, sustained ERK2 phosphorylation is required for NSC59984-induced degradation of mutant p53 protein, and depletion of mutant p53 contributes to the restoration of p53 pathway. NSC59984 induces MDM2 phosphorylation that correlates with ERK2 phosphorylation. The effect of NSC59984 on MDM2 phosphorylation is blocked by U0126 and knockdown of ERK2. NSC59984-mediated mutant p53 protein degradation is inhibited by MDM2 knockdown, and enhanced by MDM2 overexpression. Thus, ERK2-dependent MDM2 phosphorylation is a major determinant of NSC59984-mediated mutant p53 degradation. We investigated the role of ROS in the effect of NSC59984 on ERK2 phosphorylation. ROS is induced by NSC59984 and a decrease in ROS by NAC inhibits NSC59984-induced ERK2 phosphorylation, and mutant p53 protein degradation. Increased ROS by BSO treatment enhances the NSC59984 effect on ERK2 phosphorylation, mutant p53 protein degradation and restoration of p53 signaling. We conclude that ROS is required for NSC59984 to sustain ERK2 phosphorylation, which, in turn, is required for NSC59984-induced mutant p53 protein degradation via MDM2. The combination of NSC59984 and BSO synergistically induces cell death in colorectal cancer cells. ROS and ERK2 are two important factors required for NSC59984 to degrade mutant p53 protein, restore p53 signaling and induce cell death. These results provide a rationale for clinical testing of NSC59984 in tumors with high ROS.

REDUCED RPL22 EXPRESSION IS SEEN IN MDS/AML AND PREDISPOSES HEMATOPOIETIC CELLS TO LEUKEMOGENESIS

Bryan Harris[1], Jacqueline Perrigoue[1], Rachel Kessel[2], Shawn Fahl[1], Stephen Sykes[1], Amit Verma[2], and David Wiest[1]

[1]Fox Chase Cancer Center, Philadelphia, PA; [2]Albert Einstein College of Medicine, Bronx, NY.

Analysis of gene expression in CD34+ hematopoietic stem cells (HSC) from 183 MDS patients demonstrated that ribosomal protein L22 (Rpl22) was the most significantly reduced ribosomal protein gene in MDS. Interestingly, we observed that AML patients with lower expression of *RPL22* had a significant reduction in their survival (TCGA cohort, N=200, Log Rank P value<0.05). Using *Rpl22*$^{-/-}$ mice, we found that Rpl22 deficiency resulted in a constellation of phenotypes resembling MDS. Indeed, Rpl22 deficiency causes a macrocytic reduction in red blood cells, dysplasia in the bone marrow, and an expansion of the early hematopoietic stem and progenitor compartment (HSPC). Since MDS has been described as a disease originating from the stem cell compartment, we next sought to determine if the hematopoietic defects were cell autonomous and resident in *Rpl22*$^{-/-}$ HSC. Competitive transplantation revealed that *Rpl22*$^{-/-}$ HSC exhibited pre-leukemic characteristics including effective engraftment, but a failure to give rise to downstream mature blood cell lineages. Collectively, these features in the stem and progenitors predispose to leukemogenesis. We examined the potential for Rpl22-deficient HSPC to be transformed upon ectopic expression of the MLL-AF9 oncogenic fusion. Indeed, Rpl22 deficiency increased predisposition to transformation both in vitro and in vivo, in MLL-AF9 knockin mice. To determine how Rpl22-deficiency contributes to these deficiencies in HSC, we performed whole transcriptome analysis on Rpl22-/- HSC. Interestingly, alterations in genes associated with both ribosomal proteins and mitochondrial membrane components were observed. Protein synthesis was unaffected or increased in Rpl22 deficient HSCs contrary to other ribosomal deficiencies. Alternatively, we observed increased fatty acid oxidation in Rpl22 deficient HSPC. Inhibition of fatty acid oxidation mitigated retention of HSCs with Rpl22 deficiency. We found a strong correlation, in human AML, between Rpl22 and MPC2 expression in human AML, supporting the potential for Rpl22 to regulate fatty acid oxidation. Together these findings demonstrate that Rpl22 predisposes HSPC to leukemic transformation by altering metabolic properties, and is frequently reduced in patients with AML.

PKCε REGULATES REDOX BIOLOGY TO SUPPORT ACUTE MYELOID LEUKEMIA SURVIVAL

Daniela Di Marcantonio[1], Esteban Martinez E[1], Jessica Vadaketh[1], Simone Sidoli[2], Benjamin Garcia[2], and Stephen Sykes[1]

[1]Fox Chase Cancer Center, Philadelphia, PA; [2]University of Pennsylvania, Philadelphia, PA

Acute Myeloid Leukemia (AML) is a genetically heterogeneous disease characterized by the clonal expansion of myeloid precursors. This cancer affects 20,000 new patients every year in the US with an overall 5-year survival of only 25%. Therefore, new therapeutic strategies are urgently needed. New studies suggest that the redox environment of cancer cells is highly oxidized in comparison to their normal counterpart. In this context, we identified that the protein kinase PKCε is a novel regulator of AML intracellular redox biology, progression, and survival.

Using a shRNA-mediated approach, we have observed that PKCε down-modulation drastically reduces AML cell expansion and induces cell death in a genetically engineered mouse model (GEMM) of AML and in human AML cell lines. At the molecular level, we found that PKCε inhibition induces reactive oxygen species (ROS) production, especially superoxides in the mitochondria. Using a non-biased proteomic approach, we found that PKCε down-modulation causes AML cells to adopt a specific protein signature associated with altered ROS production and metabolism.

We identified the mitochondrial isoform of superoxide dismutase (SOD2) as an interesting target of PKCε due to its ability to metabolize superoxides into hydrogen peroxide (H_2O_2) that is ultimately neutralized into water by Catalase. Interestingly, we have seen that the combined over-expression of SOD2 and Catalase partially rescues the anti-leukemia effects induced by PKCε inhibition, suggesting that PKCε supports leukemia cell survival by managing mitochondrial superoxide production.

Collectively, these results uncover the previously unrecognized role of PKCε as a critical regulator of mitochondrial redox biology in AML, supporting cell survival and leukemia progression.

POSTER PRESENTATIONS
SESSION I

NEOANTIGEN BURDEN ASSOCIATES WITH PATHOLOGIC RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN BLADDER CANCER PATIENTS

Philip H. Abbosh, David Liu, Michael H. Johnson, Wafik El-Deiry, Elizabeth R. Plimack, Jonathan E. Rosenberg, and Eliezer M. Van Allen

Fox Chase Cancer Center, Philadelphia, PA

The basis of response to chemotherapy is incompletely characterized. The immune system is increasingly recognized as both a key player in cancer control and a druggable target. Neoantigens, which are tumor-specific peptides that can be recognized by the immune system, are important in mediating the recognition of tumor cells and their eradication by the immune system. We hypothesized that the immune system impacts pathologic response in patients undergoing cisplatin-based neoadjuvant chemotherapy (NAC) for muscle-invasive (≥pT2) bladder cancer though neoantigens present in tumors. To study this hypothesis, patient HLA-matched neoantigens were predicted using whole exome sequencing (WES) from two independent cohorts of patients treated with cisplatin-based NAC. Using Wilcoxon rank sum test, mutational load and neoantigen burden were correlated to pathologic response, which was defined in the cystectomy specimen. Mutation analysis and neoantigen prediction was performed using standard analytical pipelines. Mutation rate associated tightly with pathologic response. The relationship between neoantigen density and response maintained significance independent of the definition of response and independent of the definition of neoantigen. Additionally, mutational load was increased in tumors harboring point mutation of DNA repair genes *ATM* and *ERCC2*. Cisplatin may therefore exert tumor cell-autonomous effects through apoptotic mechanisms and a tumor cell-extrinsic effect by alerting the immune system via neoantigen presentation on dying cells. Both of these responses may be in part related to inability to repair DNA-inability to repair DNA may increase mutation burden and impair cisplatin adduct removal, thereby inducing apoptosis. Mutation rate is likely directly correlated to neoantigen burden. These findings have implications in application of NAC alone or in combination with immunotherapeutic agents and in development of antitumor vaccines or adoptive transfer strategies. Experiments are underway to more directly characterize the impact of the immune system in the response to cisplatin in clinical samples and mouse models.

FOXOS SUPPRESS LIPID PEROXIDATION TO PROMOTE AML PROGRESSION AND CHEMOTHERAPY RESISTANCE

Turan Aghayev, Chun Zhou, Alyssa Klein, Esteban Martinez, Claudia Scholl, Stefan Fröhling and Stephen Sykes

Fox Chase Cancer Center, Philadelphia, PA

Over 30% of acute myeloid leukemia (AML) patients do not respond to first line chemotherapy and a significant portion of patients that do initially respond subsequently relapse with a therapy resistant AML indicating that AML cells either rapidly evolve or inherently possess mechanisms for evading standard chemotherapeutic approaches. We recently discovered that the FOXO family of transcription factors, which have been traditionally considered tumor suppressor genes, actually support AML cell survival and the differentiation blockade. Previous studies have shown that, in variety of cell types, FOXOs influence the intracellular redox environment by suppressing the production of reactive oxygen species (ROS). Therefore, using fluorogenic probes that detect either total intracellular ROS content (CellRox) or superoxide production (MitoSox), we found that shRNA-mediated inhibition did not affect total levels of intracellular ROS or superoxide. However, using a lipid peroxidation sensor (BODIPY® 581/591 C11), we did observe that two distinct FOXO3-targeting shRNA alter both homeostatic and stress-induced levels of lipid peroxides in AML cells. Consistent with this data, we also found that AML cells treated with a chemical inhibitor of FOXOs (AS1842856) display increased steady-state levels of intracellular lipid peroxides as well as increased signs of differentiation (CD11b and morphological changes) and death (Annexin V staining). Both basic and clinical studies have shown that anthracyclines, such as Daunorubicin (DNR) induce lipid peroxidation, however, the role of lipid peroxidation in chemotherapy effectiveness is largely unknown. We have observed that shRNA-mediated inhibition of FOXO3 enhance DNR-mediated AML cell death whereas enforced expression of FOXO3 protects human AML cells from DNR cytotoxicity. Collectively, these results suggest that FOXOs are critical mediators of AML progression and chemotherapy resistance in part by directly regulating intracellular lipid peroxide levels.

INVESTIGATING THE ROLE OF PALLADIN ISOFORMS IN PANCREATIC DUCTAL ADENOCARCINOMA ASSOCIATED DESMOPLASIA

Jennifer Alexander[1,2] and Edna Cukierman[1]

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Pancreatic Ductal Adenocarcinoma (PDAC) is an aggressive malignancy that claims 95% of patient lives in less than 5 years after diagnosis. A challenge in treating PDAC involves its unique fibrous-like stroma (desmoplasia) that encapsulates the tumor, thereby limiting therapeutic perfusion. Desmoplasia is initiated by activated cancer associated fibroblast (CAFs) which produce a TGFβ-dependent highly-dense, collagen-rich extracellular matrix (ECM). This remodeled desmoplastic ECM (D-ECM) fuels a vicious cycle of desmoplastic dissemination by altering physical/structural and biochemical pathways in neighboring naïve fibroblasts that ultimately results in an enriched CAF population. Paradoxically, absence of D-ECM induces an even more aggressive cancer progression, whereas a homeostatic "normal" stroma (N-ECM) shifts the cycle to restrict tumor growth and invasion. Although the underlying biology remains unclear, these stromal features seem to be driven by cytoskeletal rearrangements. To better understand the participation of cytoskeleton dynamics as a potential mechanism to restore the tumor-suppressive properties of N-ECM, we focused on the actin cross-linker palladin. While palladin is identified to have an important role in PDAC desmoplasia, the contribution of specific isoforms remains elusive.

Using an *in vivo*-mimetic 3D fibroblast-derived ECM model, we explored the isoform-specific role of palladin during D-ECM production and during D-ECM-induced CAF phenotype in naïve cells via quantitative immunoblots, real-time polymerase chain reaction, and detailed microscopy analyses. We have obtained preliminary data suggesting that two of the major palladin isoforms (iso3 and iso4) correlate with TGFβ-dependent and desmoplasia induced fibroblastic conversion into CAFs. Additionally, elevated expression of iso4 promotes iso3 expression as well as the known fibroblastic activation marker, alpha smooth muscle actin (α-SMA). These findings suggest that palladin iso3 and iso4 are required for desmoplastic onset and D-ECM-induced fibroblastic activation. Together, these results propose manipulation of specific palladin isoforms could signify a strategy to restore a normal tumor-suppressive stroma in PDAC.

CHARACTERIZATION OF A NOVEL FILAMENTOUS ASSEMBLY OF THE NUCLEOTIDE BIOSYNTHETIC ENZYME INOSINE MONOPHOSPHATE DEHYDROGENASE

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Inosine monophosphate dehydrogenase (IMPDH) is a rate-limiting enzyme involved in purine nucleotide biosynthesis, which is an essential process for proliferating cells. In addition, IMPDH is an important therapeutic target for immunosuppression as well as for treatment of Hepatitis C infections. Interestingly, it has been discovered that this enzyme is capable of assembling into cytoplasmic filamentous structures. Mammalian cells do not typically assemble IMPDH filaments under normal in vitro culture conditions, but they can be triggered to assemble by reducing the availability of precursors for purine nucleotide biosynthesis. Purified IMPDH assembles into filaments *in vitro* in the presence of its allosteric regulator ATP, which binds to the CBS domain of IMPDH. However, the biological role of IMPDH filament assembly is currently unclear. Using electron microscopy in conjunction with enzymatic activity assays on purified human IMPDH2 protein, we have demonstrated that assembled IMPDH is enzymatically active. Inhibition of IMPDH activity at high concentrations of the allosteric inhibitor GTP, which also binds to the CBS domain, leads to filament disassembly. In addition, we have discovered that ATP can rescue GTP inhibition of filament assembly and IMPDH activity. Together, these results support our hypothesis that there is competitive binding between GTP and ATP to the CBS domain of IMPDH. ATP appears to promote catalytically active filaments whereas GTP binding promotes filament disassembly and loss of catalytic activity. Filament assembly therefore appears to be an adaptation to suppress feedback inhibition by GTP, the downstream product of the biosynthetic pathway. Filament assembly, therefore, may allow the accumulation of greater GTP nucleotide pools to support increased nucleotide demand. These findings may lead to novel treatment options for proliferative diseases such as cancer.

FUNCTIONAL ROLE OF PAK1/ERK SIGNALING IN RAC-RELATED DISEASES

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The RAS/MAPK signaling pathway regulates key cellular processes, including proliferation, differentiation, and survival. Somatic mutations of the RAS pathway are found in 30% of human cancers, including pancreatic, colon, thyroid, lung and melanoma. Furthermore, germline mutations in this pathway, collectively termed Rasopathy syndromes, cause abnormal development. Affected patients often present with cognitive defects, heart problems, facial aberrancies, skin abnormalities and in some cases a predisposition to cancer.

As p21activated kinases (Paks) have been shown to be required for the activation of ERK by Ras, we evaluated Pak1 and its activator Rac1 as potential therapeutic targets in both Rasopathies and in melanoma.

Using a zebrafish model, we first asked if the phenotypic consequences of Ras pathway overactivation during development could be blocked by Pak small molecule inhibitors. Introducing gain-of-function genes such as $BRAF^{WT}$, $BRAF^{V600E}$, $KRAS4A^{G12V}$ and $Rac1^{P29S}$ into one-cell zebrafish embryos produced a Rasopathy phenotype, characterized by abnormal head and heart development. These phenotypes were suppressed by a Pak-specific small molecule inhibitor. pERK and pPAK levels were increased in embryos expressing $BRAF^{V600E}$, $KRAS4A^{G12V}$ and $Rac1^{P29S}$, and were restored to baseline upon addition of Pak1 or Rac1 small molecule inhibitors. We then studied the effect of these small molecule inhibitors in human malignant melanoma cell lines bearing activating mutations in BRAF, PREX (an activator of Rac1), and/or RAC1. Treatment with PAK or RAC inhibitors decreased the migration and proliferation of cells with mutant PREX or RAC1 mutations, but not those with BRAF mutations; the reverse was true when these cells were treated with a BRAF inhibitor. These data suggest that Pak1 might serve as a useful therapeutic target in PREX or RAC1driven cancers such as malignant melanoma.

A PIPELINE FOR DEVELOPING A NOVEL PREDICTIVE TOOL TO CLASSIFY VARIANTS OF UNCERTAIN SIGNIFICANCE IN LYNCH SYNDROME

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Lynch syndrome (LS) highly predisposes individuals and their families to an increased risk of colorectal and other cancers. LS has been attributed to germline mutations in DNA mismatch repair (MMR) genes: *MLH1, MSH2, MSH6*, *PMS2*. Mutations in the *MLH1* and *MSH2* genes account for the majority of detectable mutations in LS, with more infrequent mutations in *MSH6* and *PMS2*. Clinical diagnosis of LS helps direct the management of the disease and risk assessment for future cancers in the family.

A major challenge is the determination of variant pathogenicity in MMR genes, particularly those that code for missense mutations. Variants of unknown significance (VUS) present an enormous challenge to effective genetic counseling of LS families. There are generic and MMR gene-specific VUS predictors. However these are limited in their predictive abilities and lack experimental validation. Our aim is to develop a predictive tool with a novel feature of experimental validation of variants in the training set.

We have developed and applied a pipeline to perform experimental validation of VUS in MMR genes. MMR genes with introduced VUS were assessed *in vitro* in comparison to wild type genes for the effect of the VUS on steady state protein expression, localization, and functionality in inducing DNA damage checkpoints and influencing cell survival following treatment with a panel of DNA-damaging agents. We then assessed a panel of VUS in *MSH2, MLH1* and *PMS2* in HEK293 kidney cells and colorectal cancer (CRC) cell lines bearing deletions in the MMR genes being tested.

This mid-throughput pipeline allowed effective validation of the functional consequences of each VUS, generating additional data to enhance the efficacy of gene-specific predictors for the LS genes that we are developing. Such tools may greatly benefit the assessment of MMR gene variants identified through genetic testing and for genetic counseling of LS families.

SELF-CONTROL, CONTEXTUAL VARIABLES, AND POOR HEALTH HABITS: UNDERSTANDING CANCER RISK BEHAVIORS

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Self-control failure is a potent predictor of unhealthful behaviors such as drinking and smoking and a risk factor for addiction and cancer risk. Although research examining self-control failure among addicted individuals is growing, less is known about non-addicted people who fail at controlling health behavior. Young adults such as college students are a particularly important group to examine, as precautions taken in young adulthood may affect disease risk more than adult health behaviors. This study seeks to advance current theories of self-control and their association with cancer risk behaviors. Cancer risk behaviors in this study include two behavioral categories: substance use (i.e. drinking alcohol, smoking cigarettes, and smoking marijuana), and overeating. Questionnaire data collected from 615 college student participants who completed well-validated self-report measures were analyzed. Structural Equation Modeling was used to confirm a multi-dimensional latent factor model of dispositional self-control. The multi-dimensional dispositional self-control factor predicted substance use and overeating as hypothesized, suggesting that those with higher perceived self-control are less likely to engage in substance use or overeating compared to those with lower control. Contextual variables, namely stress, fatigue, and negative mood, were predicted to moderate the relationship between latent dispositional self-control and cancer risk behaviors. Moderation findings suggested that self-control may be less efficacious in contexts involving high demands (i.e., high stress, high fatigue, and negative mood), compared to contexts involving low demands (i.e., low stress, low fatigue, and positive mood). This pattern was observed with high consistency across three moderators and with two different types of cancer risk behaviors. This study is one of the first to examine the interactive effects of variables reflecting the state of individuals with dispositional control. Furthermore, by identifying the impact of modifiable contextual factors including stress, mood, and fatigue, the study offers groundwork to advance cancer risk behavior interventions.

TWO NOVEL LONG NON CODING RNAS IDENTIFIED AS REGULATORS OF CANCER PROGRESSION IN LUMINAL AND TRIPLE NEGATIVE BREAST CANCER

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Long non-coding RNAs (lncRNAs) perform essential biological functions via predominantly unexplored mechanisms which include epigenetic regulatory mechanisms, X inactivation, and RNA processing. In recent years, they have been identified as regulators of the cell cycle, apoptosis, and DNA damage among other processes that if deregulated, may lead to cancer. LncRNAs act as proto-oncogenes, tumor suppressor genes, and drivers of metastatic transformation at the transcriptional, post-transcriptional, and epigenetic levels. They have been reported to have key roles in cancer prevention, diagnosis, and treatment; thus identifying and detecting specific lncRNAs involved in cancer is essential to understanding, preventing and reversing cancer development. We have identified 42 differentially expressed lncRNAs from which we have focused on two of these novel lncRNAs for further studies. They are lncBHLHE and lncEPCAM, which we hypothesized have tumor suppressing and tumor promoting roles, respectively, in the context of luminal and triple negative breast cancer (TNBC). The cancer phenotype was evaluated by studying proliferation, apoptosis, migration and invasion changes after overexpression of these lncRNAs. The results obtained thus far are uncovering their role in carcinogenesis. Importantly, these lncRNAs are also expressed in breast cancer tissues and ongoing xenograft studies in female SCID mice will confirm their biological role in breast cancer progression. To understand their potential regulatory role, reverse transcriptase PCR was conducted to determine if the overexpression of lncBHLHE and lncEPCAM affects *Cis* regulation in luminal and TNBC cell lines. Positive results on *Cis* regulation are being expanded using chromatin isolation by RNA immunoprecipitation to determine their genome wide regulatory role.

These results shed light on two novel lncRNAs which show tumor suppressing and tumor promoting features in breast cancer development. The significance of this work lies in the fact that these previously unidentified lncRNAs may act as key regulators of breast differentiation, cancer initiation and progression.

KINOME ACTIVATION SIGNATURE TO PREDICT PLATINUM RESISTANCE IN HIGH GRADE SEROUS OVARIAN CARCINOMA

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Patients with high-grade serous ovarian carcinoma (HGS-OvCa) are expected to respond to primary therapy with platinum and taxane, however a select population will develop platinum resistance within 6 months, leading to a worse prognosis. Currently, there are no biomarkers to predict platinum resistance, nor any treatment that can prolong survival once platinum resistance is developed. Protein kinases (collectively termed “kinome”) represent attractive therapeutic targets due to their established roles in cancer signaling and the availability of drugs to inhibit their action. Importantly, the kinome signaling networks that promote chemotherapy resistance in HGS-OvCa are largely undefined. With the use of protein kinase-capture beads and mass spectrometry to monitor activated protein kinases from tumor cells, we have begun to identify kinases unique to patients with platinum resistant cancer. To date, we have defined kinome activation signatures for a large cohort of platinum-resistant and platinum-sensitive HGS-OvCa patient tumor samples, as well as a number of patient derived xenografts (PDX) tumors. Importantly, we have identified a number of kinases that exhibit elevated kinase activity in patient tumors that display rapid resistance to cisplatin, which we believe represent very promising drug targets. Using this proteomics technique, we have also evaluated the response of the kinome to platinum damaging agents in HGS-OvCa cell lines and defined the fraction of the kinome activated by platinum therapy. Currently, we are evaluating combination therapies targeting kinases active at baseline in platinum-resistance or induced by platinum treatment in HGS-OvCa cell lines. Our studies are highly translational in nature and provide a novel opportunity to identify biomarkers of platinum resistance, as well as facilitate the discovery of new kinase inhibitor therapies for the treatment of chemotherapy-resistant HGS-OvCa.

A SECONDARY METAL BINDING RESIDUE IMPORTANT FOR ACTIVITY IN RETROVIRAL INTEGRASES

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Retroviral integrases (IN) catalyze the insertion of viral DNA into host cell DNA through two reactions, both of which require divalent metal ions. The native enzyme uses two Mg(II) ions held within the catalytic core domain of the enzyme, the enzyme is also active *in vitro* with Mn(II). Across all retroviruses, these metal ions are coordinated by the "DDE motif" in the enzyme, the phosphate backbone of the DNA, and filled in with water residues. It is likely that secondary residues play a role in metal binding as well, as demonstrated by differences in some active site inhibitors when tested against enzymes bound to Mg or Mn. These differences are not consistent among integrases from different viruses. Herein, we modeled the core of HIV IN and avian sarcoma virus (ASV) IN, to find differences in H-bonds in the core domain of these enzymes, we then mutated ASV IN in order to produce at substitution in the corresponding HIV IN residue, and studied their activity in a DNA substrate cutting assay. One substitution of interest, C125N, exhibited activity that was distinguishable from the WT IN in this assay. Both the site of cutting and the metal preference were affected. Crystallographic analysis of this substituted enzyme are underway to determine the structural implications of this residue on the active site.

ANTI-CANCER DRUGS CURAXINS INHIBIT FACT ACTION DURING POL II TRANSCRIPTION

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Human FACT (factor activating chromatin transcription) is a multi-functional protein complex that has histone chaperone activity and plays critical roles in multiple vital cellular processes (cell differentiation, DNA replication, transcription and cancer development). FACT is also a target for recently developed anticancer drugs curaxins. Previously we have proposed a model of FACT action during transcription through chromatin suggesting that FACT transiently interacts with the exposed DNA-binding surface of the H2A/H2B dimer and thus facilitates transcription and nucleosome survival during this process. In this study, the hydroxyl radical footprinting revealed that curaxins induce DNA uncoiling and exposing the H2A/H2B dimer surface in the nucleosome, providing a high-affinity site for FACT binding. As a result, curaxin-containing nucleosomes compete FACT away from the transcribed DNA regions, inhibiting FACT-dependent Pol II transcription *in vitro*. Consistently, analysis of ChIP-Seq data on SSRP1 (FACT subunit) shows that curaxins induce redistribution of FACT from the transcribed chromatin regions to other genomic loci in cancer cells. Taken together, our results reveal the mechanism in anti-cancer function of curaxins. Curaxins act through redistribution of FACT and inhibition of FACT-dependent Pol II transcription. Our study identifies FACT domains as a potential anti-cancer drug targets.

FREQUENT BRCA2 SOMATIC MUTATIONS IN COLORECTAL CANCER PATIENTS WITH MICROSATELLITE INSTABILITY (MSI)

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Microsatellite instability (MSI) is a hallmark of mismatch repair (MMR)-deficient cancers including colorectal cancer (CRC). MSI represents 15% of CRCs as a result of either epigenetic silencing of MLH1 or mutations in one of the MMR genes: MLH1, MSH2, MSH6 and PMS2. MSI tumors have a better prognosis than microsatellite stable (MSS) tumors while responding differently to treatments. Deficient MMR can lead to deficient DNA double-strand-break repair. We recently reported a high mutation rate (50%) in BRCA2 in 26 MSI-High vs. 558 non-MSI-High CRCs profiled by Caris Life Sciences. We hypothesized there might be a pattern with specific BRCA2 mutations in MSI-H CRCs targeting the coding microsatellites in BRCA2. We further investigated functional mutation patterns in BRCA2 in both MSI-H and MSS groups. Of 1104 profiled CRCs in the COSMIC v73 database, somatic BRCA2 mutations were mapped for 101 MSI-High versus 916 MSS CRCs. MSI-High CRCs showed a significantly higher mutation rate in BRCA2 as compared to MSS (38% vs 6%, $P<0.0000001$). A higher rate of damaging mutations (42% vs. 2%, $P< 0.0001$) and a relatively distinct pattern of protein mutation distribution could be clearly mapped in MSI-High CRCs versus the MSS group. We found that specific mutations in coding microsatellites of BRCA2 can be impacted by MMR defects. We found 72 unique BRCA2 mutations in MSI-H CRCs not previously seen in either breast cancer or pancreatic cancer as reported in COSMIC v73. However, using the BIC database we detected 5 BRCA2 deleterious mutations reported as germline mutations in breast cancer. We used the consensus result from five predictors and available 3-D structural information to predict deleterious properties of mutations including damaging BRCA2 protein structure and disruption of interactions with partner proteins including DSS1 and RAD51. Targeting BRCA2 mutations in MSI tumors and using the concept of synthetic lethality might be effective in BRCA-deficient CRCs with MSI.

NEDD9 DEPLETION IN MURINE MODEL PROMOTES MORE AGGRESSIVE LUNG CANCER PHENOTYPE

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Non-small cell lung cancer (NSCLC) has a low survival rate, with metastasis contributing to the vast majority of deaths. Upregulation of scaffolding protein NEDD9, a pro-metastatic factor, has been reported in a large subset of lung cancers and other malignancies. Further, NEDD9 has been identified as a scaffold for intermediates in both integrin and receptor tyrosine kinase signaling cascades. Therefore, depletion of NEDD9 was hypothesized to be likely to slow lung tumor development, a result obtained by some publications with NEDD9 shRNA knockdown xenografts. In addition, our laboratory previously reported that constitutive, whole body NEDD9 null genotype significantly reduced tumor growth in two mammary tumor models. In contrast, we have now observed a similar NEDD9 null genotype greatly accelerated tumor growth in a conditional knockout Kras/p53 mouse model for NSCLC. Further, NEDD9 null NSCLC tumors exhibited higher invasive capacity, including direct invasion to the heart and thymus, and a higher proliferation rate compared to the NEDD9 wt genotype. These suggest NEDD9 action in the tumor microenvironment selectively influencing NSCLC pathogenesis, or tumor signaling to compensate for absence of NEDD9 from the time of tumor initiation (versus shRNA knockdown in existing NSCLC cell lines). Ongoing experiments are eliciting the mechanism of NEDD9 action in NSCLC.

INDUCTION OF INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE (IMPDH) FILAMENTS DURING T CELL ACTIVATION

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The formation of macromolecular protein assemblies can play important biological roles through coordination of multiple enzyme activities, facilitation of conformational changes, and regulation of enzyme catalysis. We have identified a novel filamentous macromolecular structure that is induced during T cell activation. These filamentous structures contain the *de novo* nucleotide biosynthesis enzyme inosine-5'-monophosphate dehydrogenase (IMPDH). IMPDH carries out the rate-limiting, NAD^+-dependent conversion of inosine-5'-monophosphate (IMP) to xanthine monophosphate during purine biosynthesis. Activation of murine splenic T cells with anti-CD3ε and anti-CD28 antibodies results in a vast induction of IMPDH protein expression, accompanied by the formation of IMPDH-containing filaments. Since proliferating T cells are highly dependent on *de novo* nucleotide biosynthesis pathways to provide nucleotide precursors for signaling, growth, and division, we hypothesize that these IMPDH filaments form to facilitate purine nucleotide biosynthesis. We are currently examining whether or not IMPDH filaments are necessary for T cell proliferation. We have also determined that IMPDH filaments are more prevalent in activated $CD8^+$ cells than in activated $CD4^+$ cells. We hypothesize that this reflects different metabolic requirements for these T cell populations and plan to explore this further by examining IMPDH filament formation in other T cell subsets.

THE ROLE OF IL-23 IN THE DEVELOPMENT OF ATHEROSCLEROSIS

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Atherosclerosis is lipid-driven chronic inflammatory disease of the arterial wall mediated by innate and adaptive immune responses. Inflammation promotes the development of atherosclerotic plaques. Cytokines are soluble mediators of inflammation and important players in the pathogenesis of atherosclerosis.

IL23, a cytokine of IL6/IL12 cytokines superfamily, was shown to be pathogenic in various inflammatory diseases. IL23 regulates the production of IL17 and IL22 by T helper IL17 producing (Th17) cells, innate lymphoid cells of type 3 (ILC3), γδ T cells and also controls myeloid cells activation. Multiple reports demonstrated increased levels of IL23 in atherosclerosis, pointing out to possible pro-inflammatory pro-atherogenic role of this cytokine. Therefore, we decided to address the role of IL23 in atherosclerosis using *Il23p19 and Il23(R)* receptor deficient mice. Surprisingly, atherosclerosis prone, *Ldlr*$^{-/-}$ mice transplanted with *Il23p19*$^{-/-}$ or *Il23r*$^{-/-}$ bone marrow and fed with Western diet (WD) for 14 weeks demonstrated acceleration of atherosclerosis progression, which was characterized by increased accumulation of various hematopoietic cells in the aortas. Analysis of cytokine production unexpectedly revealed no changes in IL17A and IFNγ production by CD4 T cells in the aortas, while the production of both cytokines was downregulated in the intestine of *Il23p19*$^{-/-}$*->Ldlr*$^{-/-}$ mice. Moreover, we found enhanced expression of a pro-inflammatory molecule Osteopontin (OPN) in aortas of *Il23p19*$^{-/-}$ *->Ldlr*$^{-/-}$ mice compared to *wt->Ldlr*$^{-/-}$ controls. Based on our preliminary findings, we hypothesize that IL23-IL23R signaling may control the inflammation in atherosclerosis by directly suppressing expression of the pro-inflammatory molecule osteopontin (OPN) from myeloid cells in aortas.

Cardiovascular diseases including atherosclerosis represent a growing healthcare burden in developed countries. We have discovered a new role of the cytokine IL23 in atherosclerosis development, allowing us to put forward the exciting proposition that IL23 actually may play an immunoregulatory role in conditions of hypercholesterolemia and thus protect from atherosclerosis.

THE ROLE OF STEROL METABOLITES IN REGULATING GROWTH OF EGFR/KRAS-DEPENDENT TUMORS

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Cells transformed by oncogenic EGFR or RAS require elevated level of intracellular cholesterol, suggesting a possibility for metabolic interference.

We identified SC4MOL and NSDHL, two enzymes in the cholesterol pathway and their substrates, meiosis activating sterols (MAS), as critical regulators of receptor signaling and trafficking in normal development and in cancer. Our prior studies showed that NSDHL and SCMOL deficiency causes accumulation of MAS in the cells and also leads to an inhibition of cell growth and proliferation.

Further investigation of the mechanism of MAS activity and possibility of using NSDHL as a target in cancer revealed that NSDHL inactivation *in vivo* in adult keratinocytes expressing $KRas^{G12D}$ antagonized growth of skin tumors, while having little effect on normal skin. Loss of NSDHL activated liver X receptor (LXR) α and its transcriptional targets, causing the decrease of intracellular cholesterol level. Importantly, EGFR signaling opposed LXRα effects on cholesterol homeostasis, while an EGFR inhibitor synergized with LXRα agonists in killing cancer cells.

Given the importance of cholesterol homeostasis for the pancreatic cancer progression, we suggested that targeting cholesterol homeostasis regulation, by inhibition of SC4MOL or NSDHL, or activation of LXRα by sterol metabolites can be an effective strategy against pancreatic cancer with activated EGFR-KRAS signaling. Our preliminary data on the mouse model of pancreatic cancer in *Pdx1-Cre/LSL-KrasG12D/Tp53f/f* mice and on several human pancreatic cancer cell lines confirmed high dependence of cancer cells on the level of intracellular cholesterol. Further analysis of the *Pdx1-Cre/LSL-KrasG12D/Tp53f/f* mice model with *Nsdhl* conditional knockout will reveal the precise role of sterol metabolites in pancreatic cancer progression.

P53 REPRESSES PYRIMIDINE CATABOLIC GENE *DIHYDROPYRIMIDINE DEHYDROGENASE* (*DPYD*) EXPRESSION FOLLOWING THYMIDYLATE SYNTHASE (TS) INHIBITION

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Nucleotide metabolism in cancer cells can influence malignant behavior and intrinsic resistance to therapy. Here we describe p53-dependent control of the rate limiting enzyme in the pyrimidine catabolic pathway, dihydropyrimidine dehydrogenase (DPYD) and its effect on pharmacokinetics of and response to fluorouracil (5-FU). Using *in silico*/chromatin-immunoprecipitation (ChIP) analysis we identify a conserved p53 DNA-binding site (p53BS) downstream of the *DPYD* gene with increased p53 occupancy following 5-FU. Histone H3K9 acetylation marks at the *DPYD* promoter are diminished concomitantly with reduced expression of DPYD mRNA and protein in a p53-dependent manner. Notably we find that the P72 allele of *TP53* suppresses DPYD expression more than the R72 *TP53* allele following 5-FU treatment in mouse embryo fibroblasts. Mechanistic studies reveal inhibition of DPYD expression by p53 is augmented following thymidylate synthase (TS) inhibition by 5-FU, methotrexate (MTX), raltitrexed and TS siRNA in cancer cells. DPYD repression by p53 is dependent on DNA-PK and ATM-signaling since pharmacologic targeting of these kinases reverses the transcriptional repression of DPYD by p53. Mice lacking *TP53* in their livers have increased conversion of 5-FU to 5-FUH_2 in plasma and elicit a diminished 5-FU therapeutic response in syngeneic colorectal tumor xenografts as compared to mice with an intact *TP53* allele consistent with increased DPYD-activity. Our data suggest that p53 plays an important role in controlling pyrimidine catabolism through repression of DPYD expression, particularly following metabolic stress imposed by nucleotide imbalance. The findings have implications for the toxicity and efficacy of the cancer therapeutic 5-FU.

RPL22 LOSS IN HIGH-RISK PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Acute lymphoblastic leukemia (ALL) is the most common childhood cancer with a five-year survival rate exceeding 85%. Nevertheless, there are aggressive subtypes that frequently relapse and have low survival rates. Thus, it is crucial to develop new novel therapeutic interventions to improve outcomes for patients with high-risk ALL.

Ribosomal protein L22 (*Rpl22*) plays a critical role in regulating normal lymphocyte development, and also serves as a haploinsufficient tumor-suppressor, whose inactivation is correlated with poor survival in pediatric T-ALL. Herein we wished to distinguish the ALL subtypes in which *Rpl22* is inactivated, identify co-regulated molecular pathways, and determine if those pathways can be exploited therapeutically. By analyzing publically available genomic data, we determined that *Rpl22* inactivation occurs most frequently in the two most aggressive ALL subsets; early T-cell precursor ALL and hypodiploid ALL, providing an explanation for our previous finding that *Rpl22* inactivation was inversely associated with survival. *Rpl22* inactivation is associated with induction of genes involved in endoplasmic reticulum (ER) stress response. We had previously determined that *Rpl22* inactivation promotes leukemogenesis by activating the NF-kB/Lin28B signaling axis. We have now found that activation of NF-kB, and transformation in acute in-vitro assays, is dependent upon ER-stress signaling. This suggests that targeting ER-stress responses may represent an effective therapeutic strategy for *RPL22* mutant ALL patients. Indeed, we have discovered that *Rpl22* mutant T-ALL lines are sensitive to pharmacologic agents that induce ER-stress signaling, including Bortezomib (Velcade). We are now in the process of establishing patient derived xenografts in order to assess the efficacy of Bortezomib on T-ALL with low *Rpl22* expression.

In summary, these findings reveal that *Rpl22* inactivation is enriched in aggressive T- and B-ALL subtypes and that ER-stress signaling is an attractive and promising new target for treating this exceptional group.

IL17RB SIGNALING IN INTESTINAL INFLAMMATION AND CANCER

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Members of the interleukin-17 (IL-17) family of cytokines and IL-17 receptor (IL-17R) signaling have recently emerged as critical players in inflammation and tumor progression. It has been recently demonstrated that the expression IL-17B and IL-17E is upregulated during acute and chronic inflammation and immune responses in various tissues, including the colon. However in colitis and colon cancer, the role of interleukin-17 receptor B (IL17RB), which IL-17B and IL-17E both bind to, still remains elusive. Also, it is not known whether IL-17RB signaling is important in epithelial or myeloid cells. We constructed two kinds of IL17RB conditional knockout mouse models; IL17RB f/f villin Cre+ mice, where IL17RB is specifically knockout in colon epithelial cells, and IL17RB f/f LysM Cre+ mice where the deletion occurs specifically in myeloid cells-macrophages and neutrophils. We induced colitis in both models by one cycle of dextran sulfate sodium (DSS). No difference was found on IL17RB f/f villin Cre+ mice in severity of colitis. But surprisingly, IL17RB f/f LysmCre+ mice began to lose weight later than their WT counterparts and their colons were consistently longer, which together with improved histological score indicated less severe colitis in IL17RB f/f LysmCre+ mice, indicating colitis-promoting role of Il17RB expressed in myeloid cells.

In azoxymethane induced colon cancer model, we observed less tumors in 17RB f/f villin Cre+ mice. These data suggested that IL17RB in colon epithelial cells plays a vital role in colon cancer and knockout of IL17RB inhibits tumor growth. Our studies have further revealed that IL-17RB controls specific inflammatory gene program in colitis and cancer Overall, IL-17RB signaling in different cellular compartments, namely epithelial and myeloid cells, controls colitis and colitis-associated cancer development that represents a novel inflammatory mechanism driven by IL17 family member.

MEASURING HISTONE ACEYLATION DYNAMICS DURING DROSOPHILA DEVELOPMENT VIA MASS SPECTROMETRY ANALYSIS

Ryan A. Henry, Tanu Singh, Yin-Ming Kuo, Alison Biester, Abigail O'Keefe, Sandy Lee, Andrew J. Andrews, and Alana M. O'Reilly

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Histone modification plays a major role in regulating gene transcription and ensuring the healthy development of an organism. Numerous studies have suggested that histones are dynamically acetylated during developmental events to control gene expression levels in a temporal and spatial manner. However, the study of histone acetylation dynamics using currently available techniques is hindered by the sheer number of available sites for acetylation: on the histone octamer, there are over 100 lysines that serve as potential sites for acetylation. This creates a difficulty in simultaneously measuring acetylation on each of these sites in an efficient, quantitative manner. To address these problems, we have developed a methodology that allows us to combine high throughput mass spectrometry-based histone analysis with *Drosophila* developmental genetics. Using this system, we are able to simultaneously monitor the acetylation changes on multiple histone residues in *Drosophila* throughout their lifespan and in a tissue specific manner. In this way we are able to characterize how histone acetylation patterns change throughout multiple developmental stages, which in turn allows us to determine how each of these stages may be impacted by various external factors. Along these lines, we have also utilized our methodology to detect altered histone acetylation patterns in response to pharmacological agents, nutritional supplements, and environmental stresses, such as gamma-irradiation. These results demonstrate the efficacy of our combined mass spectrometry system with a *Drosophila* model system and provide insight into the effects of pharmacological and environmental agents on global histone acetylation patterns and the changes found in histone acetylation throughout development.

ANTI-TUMOR EFFECT AND DESTABILIZATION OF MUTANT P53 BY CB002, A P53-PATHWAY RESTORING SMALL MOLECULE

Liz J. Hernández-Borrero, Shengliang Zhang, David T. Dicker, and Wafik S. El-Deiry

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Tumor suppressor p53 is a master regulator of genotoxic and cellular stress signals, controlling cell fate by transcriptionally activating genes involved in DNA repair, cell cycle arrest, and apoptosis. p53 is mutated in over half of human cancers and this is associated with tumor development and chemotherapy resistance. These mutations not only prevent p53 to exert its normal tumor suppressive functions but can result in gain-of-function activity, acquiring oncogenic characteristics. Therefore, altering the stability of mutant p53 protein is an attractive therapeutic strategy in cancer cells. We investigated small molecules that modulate mutant p53 stability and restore the p53-signaling pathway. We identified a small molecule, CB002, as a candidate for restoration of the p53 pathway in mutant p53-bearing cancer cells. Three colorectal cancer (CRC) cell lines: SW480, DLD-1, HCT116-R175H and the RXF393 renal cancer cell line, were treated with different concentrations of CB002 at various time points. Cell lines exposed to CB002 showed an increase in apoptotic and cell death markers, such as NOXA/DR5 induction, cleaved caspases and PARP. CB002 decreased mutant p53 stability in structural conformation-mutant bearing cells (HCT116 R175H and RXF393). p53 (R175H) mutant protein expression was largely rescued by the co-treatment with MG132, a proteasomal inhibitor, implicating a role for the ubiquitin proteasome system. Therefore, we hypothesize that CB002 is capable of degrading mutant p53 and restoring the p53 pathway through p53 family members in colorectal cancer cells. Despite efforts in developing p53 targeted therapy, to date there are no FDA approved drugs targeting mutant p53 expressing CRC cells. This project aims to address this unmet need and overcome therapy resistance and tumor recurrence associated with current chemotherapy. Hence, our results provide an insight on effective p53 pathway activation through the use of small molecules.

IDENTIFICATION OF A CPG ISLAND METHYLATOR PHENOTYPE (O-CIMP) IN HIGH GRADE SEROUS OVARIAN CANCER

Ilsiya Ibragimova, Michael Slifker, Suraj Peri, and Paul Cairns

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Central to our current understanding of cancer is the finding that a type of cancer from a particular organ site actually consists of multiple subtypes delineated by differences in molecular alterations, biology, behavior and outcome. The definition of molecular subtypes provides insight into the biology of the cancer and importantly should allow stratification of patients towards more accurate prognosis and optimal choice of therapy for improved management overall. One type of frequent genomic alteration in tumor cells is aberrant hypermethylation of the promoter CpG island associated with transcriptional silencing of the gene. Recent studies have revealed the importance of the CpG island methylator phenotype (CIMP), defined by widespread and concordant promoter methylation of multiple genes, in different types of cancer. Most notably CIMP-positive colorectal cancers (CRCs) are associated with *BRAF* mutation and specific clinicopathologic features while CIMP-positive gliomas are diagnosed at a younger at age, are low grade and have an improved outcome. Here, we investigated high grade serous ovarian cancer (HGS OC), the most common type and deadly form of ovarian cancer, for the presence of a CIMP-positive subtype. We interrogated genome-wide DNA methylation data of 489 OC from the Cancer Genome Atlas. After filtering we identified the most differently methylated probes across 443 OC followed by unsupervised cluster analysis that revealed the presence of a CIMP-positive subgroup of HGS OC (O-CIMP). Importantly, concordant gene methylation was evident and included genes diagnostic of CIMP in CRC and other types of cancer. Examination of the clinicopathological features and molecular correlates of the CIMP-positive subgroup will strengthen the definition of this novel molecular subgroup and facilitate validation of O-CIMP.

MECHANISM AND ROLE OF IFN-γ-DRIVEN CELL DEATH IN SALMONELLA PATHOGENESIS

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Salmonella is an example of a bacterial genus that invades cells to induce pathogenesis. *Salmonella* species (especially the *S. enterica* serotypes *typhi* and *typhimurium*) cause disease in ~90 million people every year worldwide. *Salmonella* infects both phagocytic immune cells (such as macrophages), as well as non-phagocytic cells. Upon entry into cells, *Salmonella* forms a protective vacuole in which it can replicate unabated by the host innate immune response. But in macrophages, the presence of the cytokine IFN-γ ruptures the protective vacuole, releasing *Salmonella* into the cytosol, where it triggers a rapid, pro-inflammatory form of death called pyroptosis. Remarkably less is known about what happens to non-phagocytic cells upon infection by *Salmonella*. As these are the first cell types infected by *Salmonella*, understanding how they respond to this bacterium, and whether IFN-γ/pyroptosis clearance mechanisms are similarly at play in these cells as they are in macrophages, is an important objective.

Our preliminary data demonstrate that infecting epithelial and fibroblastic cells with *Salmonella* and later exposing them to IFN-γ, mimicking the environment of infection in the intestine, induces a novel form of cell death that is neither pyroptosis nor any of the other reported mechanisms of programmed cell death. Instead, our data reveals that this death likely results from an unknown secreted factor driven by actively replicating *Salmonella* in the cytosol of infected cells. We did not detect differences in bacterial burden between live and dead cells, indicating that, unlike pyroptosis, this is not a mechanism of controlling bacterial infection. We therefore hypothesize that exposure of non-phagocytic cells to IFN-γ is deleterious to the host, by at least two mechanisms: (1) by promoting influx of *Salmonella* in the cytosol of the infected cell; and (2) inducing destruction of the intestinal epithelial barrier to allow *Salmonella* dissemination beyond the gut.

POSTER PRESENTATIONS
SESSION II

PROFILING THE CANCER KINOME USING QUANTITATIVE CHEMICAL PROTEOMICS

Katherine J. Johnson, Jennifer Brown, Alison Kurimchak, and James Duncan

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Aberrant kinase signaling has been found to be a major contributing factor in the development of many types of cancers, which make them a critical part of the proteome to understand. Of the 518 known human kinases (kinome), only a small fraction are understood and targeted with inhibitors for cancer therapies because of the substantial cost that is necessary to analyze the remaining untargeted kinome. Our lab has developed a novel technique that allows us to capture the majority of the expressed kinome within a sample and rapidly quantitate global kinome signaling by using a combination of multiplexed inhibitor beads (MIBS) with LC-MS. A heavy kinome standard (HKS), consisting of a blend of heavy isotopically labeled cell lines, is mixed with either cell lysates or patient tumors to allow for quantitation across a variety of different types of samples. To develop the HKS, kinome profiling of cancer cell lines from the NCI-60 panel was carried out and five cell lines containing the most diverse kinome were chosen to be a part of the HKS. By adding the HKS to samples, we were able to identify and quantitate approximately 300 kinases in a single sample with a large percentage of the identifications being a part of the untargeted kinome. Currently we are applying this technique for profiling the top five ovarian cancer cell lines recently reported to be genomically similar to high grade serous ovarian carcinomas (Kuramochi, COV362, SNU119, OVCAR4, OVSAHO), as well as classical ovarian cancer cell lines (OVCAR3, OVCAR5) and normal ovarian cancer cell lines. Using this approach across several cancer types, we have the capability to achieve a better understanding of kinase signaling for a large portion of the kinome with the ultimate goal of providing more effective targets for cancer therapies.

DUAL INHIBITION OF AKT AND KIT PROVIDES SYNERGISTIC EFFECTS IN PRECLINICAL STUDY OF GASTROINTESTINAL STROMAL TUMOR

Phillip Zook, Harsh B. Pathak, Marya Kozinova, Martin G. Belinsky, Lawrence Gersz, Karthik Devarajan, Yan Zhou, Andrew K. Godwin, Margaret von Mehren, and Lori Rink

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Purpose: Gastrointestinal stromal tumors (GIST) generally harbor activating mutations in the receptor tyrosine kinase KIT or in the related platelet derived growth factor receptor alpha (PDGFRA). GIST treated with imatinib mesylate (IM) or second-line therapies that target mutant forms of these receptors generally escape disease control and progress over time. Inhibiting additional molecular targets may provide more substantial disease control. Recent studies have implicated the PI3-kinase/AKT pathway in the survival of IM-resistant GIST cell lines and tumors. **Experimental Design**: We performed *in vitro* and *in vivo* studies evaluating the novel combination of IM with the AKT inhibitor MK-2206 in GIST. Whole-transcriptome sequencing (WTS) of xenografts was performed to explore the molecular aspects of tumor response to this novel combination and to potentially identify additional therapeutic targets in GIST. **Results**: This drug combination demonstrated significant synergistic effects in a panel of IM-sensitive and -resistant GIST cell lines. Furthermore, combination therapy provided significantly greater efficacy, as measured by tumor response and animal survival, in IM-sensitive GIST xenografts as compared to treatment with IM or MK-2206 alone. WTS implicated two neural genes, brain expressed X-linked 1 (BEX1) and neuronal pentraxin I (NPTX1), whose expression was significantly up-regulated in combination-treated tumors compared to tumors treated with the two monotherapies. Interestingly, both BEX1 and NPTX1 have been linked to increased mitochondrial translocation of pro-apoptotic BAD and BAX proteins and ultimately enhanced cell death. **Conclusion**: These studies provide strong preclinical justification for combining IM with an AKT inhibitor as a front-line therapy in GIST. In addition, the WTS data suggests a potential mechanism for this synergy, in which the combination leads to induction of pro-apoptotic BEX1 and/or NPTX1 shifting the pro/anti-apoptotic balance towards cell death.

COMPROMISED RNF168-UBIQUITIN-53BP1 PATHWAY SIGNALING PROMOTES VIABILITY IN BRCA1 DEFICIENT CANCERS

John Krais, Andrea Bernhardy, Yifan Wang, Joseph Nacson, and Neil Johnson

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BRCA1 plays an important role in repairing DNA damage by homologous recombination (HR). HR is negatively regulated by 53BP1, a DNA damage response protein that blocks a process called DNA end resection. At sites of double stranded DNA breaks (DSBs), the E3-ubiquitin ligase RNF168 ubiquitylates histone γ-H2AX, 53BP1 then binds to the ubiquitin-γ-H2AX recruitment site. In this study, we investigated the efficiency of 53BP1 recruitment to sites of DNA damage and the impact of localization on viability in *BRCA1* mutant cancer cells.

The BRCA1 proficient cell lines MDA-MB-231, MCF7, and SUM149PT formed robust ubiquitin-γ-H2AX and 53BP1 foci in response to γ-irradiation, with >73% of cells demonstrating foci. In contrast, BRCA1 deficient cell lines SUM1315MO2, HCCC1395, and MDA-MB-436 demonstrated significantly lower levels of ubiquitin-γ-H2AX and 53BP1 foci positive cells, with <16% positive. Because cells had low levels of ubiquitin foci, we measured RNF168 levels. In quantitative RT-PCR assays, SUM1315MO2, HCC1395 and MDA-MB-436 exhibited 4.1, 2.7, and 1.9 fold lower expression levels of RNF168 compared to MDA-MB-231 cells, respectively.

We investigated the functional consequences of loss of 53BP1 foci in BRCA1 deficient cell lines. First, we overexpressed ubiquitin-γ-H2AX and measured 53BP1 foci and cell viability. Ubiquitin-γ-H2AX overexpression increased 53BP1 foci from 11% to 44%, and dramatically decreased cell viability in SUM1315MO2 cells. In contrast, overexpression of ubiquitin-γ-H2AX had no impact on viability in BRCA1 positive cell lines. Similar results were obtained with RNF168 overexpression.

In conclusion, BRCA1 deficient cells had reduced ubiquitin and 53BP1 foci formation resulting from low RNF168 expression levels. Furthermore, ectopic overexpression of RNF168 or ubiquitin-γ-H2AX restored 53BP1 foci and reduced cell viability. We predict that in a BRCA1 deficient state, loss of 53BP1 foci promotes residual levels of HR and cell viability. In future studies, we will examine the impact of 53BP1 foci formation on HR DNA repair.

INVOLVEMENT OF THE BAP1 TUMOR SUPPRESSOR IN ULTRAVIOLET RADIATION-INDUCED DNA DAMAGE REPAIR

Anna-Mariya Kukuyan, Eleonora Sementino, Craig W. Menges, Raza Zaidi, and Joseph R. Testa

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The BRCA-Associated Protein1 tumor suppressor gene (*BAP1*) encodes a deubiquitinating enzyme (DUB) that regulates many facets of cellular biology. Genetic studies demonstrated that somatic *BAP1* mutations are found in numerous cancers and that germline *BAP1* mutations lead to a cancer susceptibility disorder that predisposes carriers to various types of cancer, in particular malignant mesothelioma (MM) and both ocular and cutaneous melanomas (CM). The genetic and biochemical mechanisms by which alterations of *BAP1* predispose individuals to tumors are unknown. Recent evidence indicates that BAP1 is critical in the DNA damage repair response, suggesting that BAP1's role in tumorigenesis could be particularly important in cancers associated with environmental carcinogens such as ultraviolet light (UV) and asbestos. To investigate the role of BAP1 in DNA damage, we used shRNA to knock down *BAP1* in LP9 human mesothelial cells and human melanocytes. Cells were exposed to UV radiation and analyzed for Rad51/BRCA1 foci accumulation via immunofluorescence microscopy. Formation of foci was altered when *BAP1* was knocked down, consistent with a role for BAP1 in DNA repair. In a complementary experiment, mouse melanocytes (Melan-a) expressing shRNA against *Bap1* or control GFP were exposed to UV radiation, and clonogenic assays were performed for 10-14 days. Knockdown of *Bap1* in UV-exposed Melan-a cells resulted in diminished colony formation when compared to shGFP-expressing control cells. Additionally, comet assays demonstrated increased DNA damage in UV-exposed shBap1 cells compared to UV-exposed shGFP control cells. Significantly, these data implicate BAP1 in the DNA repair response both in mesothelial cells and melanocytes, suggesting that *BAP1* mutation carriers have an increased susceptibility to DNA damage and tumor formation caused by carcinogenic environmental factors such as asbestos fibers and UV radiation.

ADAPTATION OF THE KINOME PROMOTES RESISTANCE TO BET BROMODOMAIN INHIBITORS IN OVARIAN CANCER

Alison M. Kurimchak[1], Claude Shelton[1], Kelly E. Duncan[1], Katherine Johnson[1], Shane O'Brien[1], Lauren Fink[1], Yuesheng S. Li[1], Wayne E. Childers[2], Denise C. Connolly[1], Jonathan Chernoff[1], Jeffrey R. Peterson[1], and James S. Duncan[1]

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The bromodomain protein BRD4 is essential for growth in a subset of ovarian cancer cell lines. Small molecule BET bromodomain inhibitors (BETi) are actively being pursued in clinical trials for the treatment of a variety of cancers, however, resistance to these inhibitors occur, and the mechanisms of resistance remain poorly understood. Using a novel mass spectrometry approach that globally measures kinase signaling at the proteomic level, we evaluated the response of the kinome to targeted BET inhibitor treatment in a panel of BRD4-dependent ovarian carcinoma (OC) cell lines. Despite initial inhibitory effects of bromodomain inhibition, the majority of OC cells acquired resistance following sustained treatment with the BETi, JQ1. Through application of Multiplexed Inhibitor Beads (MIBs) and mass spectrometry, we demonstrate that BETi resistance is mediated by adaptive kinome reprogramming, where activation of compensatory pro-survival kinase networks overcomes BET protein inhibition. Furthermore, drug combinations blocking these kinases may prevent or delay the development of drug resistance and enhance the efficacy of BET inhibitor therapy.

MICRORNA-MEDIATED PATCHED SILENCING DURING MEDULLOBLASTOMA TUMORIGENESIS

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Medulloblastoma (MB) is the most common malignant brain tumor in children. MB frequently occurs among patients with Gorlin syndrome, caused by heterozygous germline mutation of *patched1* (*ptch1*), an antagonist of hedgehog signal pathway. The wildtype allele of ptch1 is often silenced in Gorlin syndrome, leading to MB tumorigenesis. However, the mechanism underlying such loss of heterozygosity of *ptch1* is still unknown. After ruling out the possibility of ptch1 gene mutation and methylation, we decided to investigate the involvement of microRNAs in silencing ptch1 gene expression in MBs. Through microRNA profiling, we found microRNA-106b-5p that highly expressed in MB cells. Enhanced microRNA-106b-5p in MEF cells significantly repressed *ptch1* gene expression, whereas inhibitor of microRNA-106b-5p rescued *ptch1* expression in MB cells, indicating the capacity of microRNA-106b-5p in silencing ptch1 gene expression. By gene mutation assay, we have further confirmed that microRNA-106b-5p repressed *ptch1* expression through binding the encoding sequence, instead of 3'-untranslated region of *ptch1* gene. The above findings demonstrate the important role of microRNA in the tumorigenesis of MB, and reveal a unique mechanism that microRNA exerts in repressing gene expression (i.e. by binding the encoding sequence). Moreover, our studies pave the road to treat MB by restoring ptch1 expression through inhibition of microRNA-106b-5p.

NOVEL MIRNA-BASED THERAPEUTIC APPROACH TO SELECTIVELY TARGET MUTANT P53 IN CANCER

Amriti R. Lulla, Margret B. Einarson, Yan Zhou, David T. Dicker, and Wafik S. El-Deiry

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Tumor suppressor p53, guardian of the human genome, is frequently mutated or functionally dysregulated in more than 50% of human tumors. p53 mutation is a later event in tumorigenesis and a number of p53 mutants have “Gain of Function” (GOF) properties which have been shown to promote invasive and more aggressive phenotypes in cancer cells. Mutant p53 has been an attractive and promising therapeutic target for advanced stages of tumors. Yet, mutant p53 has proved to be one of the most undruggable targets. Thus, there is scope for understanding the additional gain of function properties of mutant p53 and designing novel strategies to target mutant p53 and/or key gain of function pathways thereby. Mature MicroRNAs (miRNAs) are ~22nt endogenous, non-coding RNA sequences that bind to 3’UTR of their target genes and inhibit their translation. miRNA mimics are emerging therapeutics and attractive tools for mapping pathway networks. In this study, we have developed a novel functional high throughput screening (HTS) assay to identify miRNAs that selectively target mutant p53 cell lines. The HTS was performed in isogenic TP53+/+ (wild-type), TP53-/- (null) and TP53 R175H (mutant) HCT-116 colorectal cancer cell lines. Cell viability was used as the HTS read-out of our functional screen. Of the 2754 miRNA mimics screened, we have identified 22 key miRNAs that selectively target TP53 R175H (mutant) cells. Our ongoing work is directed to further validating and identifying which of these miRNA mimics selectively induce apoptosis in mutant p53 cells. In addition, we are using the similar approaches to identify miRNA mimics that synergize with frontline therapy, 5-Fluorouracil (5-FU). Our proposed therapeutic strategy is novel and unexplored, thus providing a unique opportunity for development of targeted therapy for TP53 mutant tumors.

EXPLORING STROMAL EXTRACELLULAR MATRIX AND KRAS MEDIATED INTERACTIONS ON HUMAN PANCREATIC CANCER PROGRESSION *IN VITRO*

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Desmoplastic (desmo) stroma is a prognostic marker for pancreatic cancer, yet mechanisms behind its tumorigenic effects remain poorly understood. Tumor stroma, known to act permissive to naïve cells, may exacerbate tumor progression in mutated cells, whereas normal stroma is restrictive to even aggressive cancer cells. The mechanisms involved in these responses remain unclear. Here we describe a new 3D *in vitro* model to study interactions between human pancreatic stromal extracellular matrices (ECMs) and pancreatic epithelial cells to identify mechanisms that regulate pancreatic cancer progression. Our 3D model is comprised of physiologically relevant 3D desmo and normal cell-derived matrices as well as polyacrylamide gel-based bioengineered ECMs. The influences of these cell-derived and bioengineered ECMs were tested using benign and kRAS-mutated aggressive isogenic pancreatic ductal epithelial cells. We found that physiological normal ECMs restricted the behavior of the kRAS mutated cells when compared with syngeneic permissive/desmo ECMs; indicating that a normal ECM has a protective role in pancreatic cancer.

To discern specific microenvironmental cues that drive the protective function of normal ECM, we independently modulated stiffness, topography, and biochemical composition of the original physiologic cell-derived ECM to mimic conditions of human normal or tumor pancreatic tissue. Our data suggests that the physiologic stiffness of normal pancreatic tissue as well as isotropic ECM architecture accounted for the most tumor suppressive kRAS cellular phenotype. To identify the molecular mechanism underlying tumor suppression, we examined MAPK/pERK1/2 pathway, a critical mechanotransduction pathway associated with tumorigenesis. We determined that pERK1/2 subcellular localization was drastically altered in kRAS cells incubated in different microenvironmental conditions, while total pERK1/2 levels remained unchanged. In summary, we have designed and implemented a new system to accurately manipulate tumor microenvironmental cues and study their interactions on kRas mutated cells. We anticipate that our studies will lead to a better understanding of ECM- cancer cell interactions to help identify novel biomarker for early detection or prevention of pancreatic cancer.

STRUCTURE-FUNCTION STUDIES OF THE HUMAN HETEROCHROMATIN-NUCLEAR LAMINA TETHERING PROTEIN, PRR14

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Tethering of heterochromatin at the nuclear lamina provides an organizational hub for gene-poor repetitive DNA and epigenetically silent genes. Such organization is important for overall nuclear structure and gene regulation in development and disease. The detailed mechanisms however are not fully understood. Previously, we identified a novel heterochromatin-nuclear lamina tethering protein, PRR14, that links H3K9me3-HP1-marked heterochromatin to Lamin A/C. In contrast to known nuclear envelope transmembrane-anchored proteins, PRR14 shows dynamic behavior during mitosis, and reassembles in two steps: heterochromatin reattachment in anaphase and nuclear lamina reattachment in telophase. Knockdown of PRR14 promotes release of heterochromatin from the nuclear lamina, and also causes nuclear shape defects as seen in cancer and laminopathies. PRR14 is therefore a critical factor for nuclear lamina-heterochromatin tethering and nuclear organization. Here, we study the structure and function of PRR14 in an effort to uncouple its tethering and nuclear structural roles.

PRR14 binds to the heterochromatin protein HP1 through its N-terminal domain, and to the nuclear lamina through the central region. Knockdown experiments had indicated that release of PRR14 from HP1-heterochromatin required depletion of all three HP1 isoforms (α, β, γ). We find, using co-IP and BioID-based analyses, that PRR14 can bind all HP1 isoforms, but primarily interacts with HP1α. We have now determined that a PRR14 central region of 50 contiguous amino acids is both necessary and sufficient for PRR14 nuclear lamina localization. The C-terminal domain of PRR14 is a Tantalus domain, but its function is uncertain. We have employed the BioID method for unbiased identification of binding partners of PRR14 by mass spec. Among the candidates we found were subunits of the PP2A phosphatase. Mass spectrometry also revealed numerous CDK1 phosphorylation sites on the PRR14 molecule. We suggest that mitotic disassembly and reassembly of PRR14 is regulated by means of CDK1 and PP2A.

PERSISTENT MEASLES VIRUS IN THE BRAIN AFTER RESOLUTION OF ACUTE INFECTION

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Viral infections of the central nervous system (CNS) can lead to debilitating diseases such as encephalitis, resulting in long lasting neurological defects, and often cause death. While many DNA viruses can cause latent infections within the brain, neurotropic RNA viruses are generally not considered to persist after acute infection, despite the knowledge that RNA viruses can cause neuropathology long after initial infection has resolved. The potential that RNA viruses may persist, and under some conditions, reactivate, has not been explored.

Our laboratory has shown that immunocompetent C57Bl/6 mice, engineered to express a measles virus receptor in CNS neurons, can functionally resolve MV (a negative sense ssRNA virus) from the CNS with no lasting neurological consequences. However, viral RNA and mRNA persist in the brains of these mice for greater than 90 days after viral challenge. To further understand how this virus evades sterile clearance from neuronal populations, we depleted the adaptive immune response from these mice, which led to increased levels of MV RNA, mRNA, and protein within the CNS. Further, we have characterized the persistence of MV in a variety of KO mouse models, and have identified CD8+ CNS T resident memory cells as a likely effector in preventing long-term viral reactivation. Currently, we are working toward identifying the mechanisms used by these CD8s to prevent viral reactivation and subsequently understand how changes in these control mechanisms may lead to pathogenesis. A better understanding of the immune factors that regulate neurotropic RNA virus persistence and reactivation may be especially relevant to inflammatory CNS diseases of unknown etiology.

CHOLESTEROL BIOSYNTHESIS REPRESENTS A THERAPEUTIC TARGET FOR HEDGEHOG PATHWAY MALIGNANCIES

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The Sonic hedgehog (Shh) signaling pathway plays a key role in cell proliferation during normal development. Aberrant activation of the Shh pathway leads to numerous human malignancies, including medulloblastoma (MB) – the most common brain tumor in children. We have previously demonstrated that constitutive activation of Shh pathway in cerebellar granule neuron precursors leads to MB. Existing targeted therapies for MB are aimed at blockade of Shh signaling through inhibition of its effector transmembrane protein Smoothened (Smo). This approach is problematic as the high doses required for tumor regression result in severe side effects and drug resistance. Recent studies revealed that activation of Smo requires interaction with cholesterol on its intracellular domain. Here, we demonstrate that MB cell-derived cholesterol is required for Shh signaling *in vivo*. Additionally, this ablation of Shh signaling by cholesterol depletion, both genetically and via cholesterol synthesis inhibitors, results in dramatic inhibition of tumor cell proliferation and allograft growth. Because cholesterol and traditional Smo antagonists act on distinct sites on the Smo protein, we hypothesized that inhibiting both sites could synergize to result in further tumor reduction and survival. Excitingly, we found that combination therapy with cholesterol inhibitor Simvastatin and a low dose of Smo antagonist GDC0449 in our MB allograft model results in a synergistic effect, decreasing MB tumor burden. This strategy offers a promising new avenue of therapy for this population of pediatric patients who cannot tolerate high doses of Smo inhibitors, along with further novel implications for other Shh-dependent cancers.

THE BRCA1-PALB2 COMPLEX IS ESSENTIAL FOR HOMOLOGOUS RECOMBINATION AND 53BP1-DRIVEN PARP INHIBTOR RESISTANCE

Joseph Nacson, Andrea Bernhardy, John Krais, Yifan Wang, and Neil Johnson

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Patients that harbor *BRCA1* germline mutations have a higher risk of developing cancer. BRCA1 likely suppresses tumor formation by promoting homologous recombination (HR) DNA repair. BRCA1 associates with a variety of partners, including CtIP and PALB2, which bind to the BRCT repeats and the coiled-coil region of BRCA1, respectively. In this study, we aimed to define the minimal region of BRCA1 required for its role in HR DNA repair and PARP inhibitor (PARPi) resistance.

We utilized the MDA-MB-436 BRCA1 null breast cancer cell line to perform a series of ectopic truncated BRCA1 add-back experiments and measured RAD51 foci formation as a marker of HR, as well as PARPi sensitivity. Overexpression of a BRCA1 protein lacking exons 12-24, that excluded the coiled-coil and BRCT domains of BRCA1, failed to promote RAD51 loading or PARPi resistance. In contrast, overexpression of a less severely truncated form of BRCA1, lacking exons 16-24, that retained the coiled-coil but lacked the BRCT domains, was able to partially restored RAD51 loading and PARPi resistance. Because the coiled-coil region of BRCA1 facilitates PALB2 complex formation, we introduced a BRCA1 missense mutation (L1407P) that specifically disrupts the ability of BRCA1-PALB2 to interact. Overexpression of BRCA1- L1407P had no impact on RAD51 foci or PARPi rescue, suggesting that the ability to interact with PALB2 was critical for RAD51 loading and PARPi resistance. Additionally, 53BP1 loss of function has previously been demonstrated to promote PARPi resistance through activating HR DNA repair. We found that 53BP1 shRNA treated cells promoted PARPi resistance only in cells that expressed BRCA1 hypmorphic proteins capable of interacting with PALB2.

In summary, we dissected the relative importance of BRCA1 domains for HR and PARPi resistance. Our findings could help stratify patients that will most likely benefit from PARPi and identify those with increased risk of developing resistance.

NESTIN EXPRESSION IS CRITICAL FOR REGENERATION OF NEURAL STEM CELLS FOLLOWING TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is a major health problem worldwide. Currently there is no effective approach to enhance brain structural repair and functional recovery after TBI. Regeneration of neural stem cells (NSCs) has been detected in the injured brain and is believed to be beneficial for brain function restoration. However, the mechanisms underlying NSC regeneration remain poorly understood. Nestin, a filament protein predominately expressed by NSCs, has long been considered a putative marker for stem cells. By using a TBI mouse model, we have recently found that NSC regeneration after TBI relies on hedgehog (Hh) signaling. Nestin expression significantly augments the output of Hh pathway activation, thereby mediates TBI-induced NSC regeneration. To achieve this, Nestin abrogates the inhibitory functions of Gli3, an antagonist of the Hh signaling pathway. These findings demonstrate that Nestin plays a critical role in regulating Hh signaling in NSCs, shedding light on molecular basis of NSC regeneration following TBI. Our studies provide the rationale to utilize Nestin as a novel therapeutic target to treat TBI, through promoting NSC regeneration.

THE ROLE OF IL-27 RECEPTOR SIGNALING IN THE DEVELOPMENT OF ABDOMINAL AORTIC ANEURYSM

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Abdominal aortic aneurysm (AAA) is a cardiovascular pathology characterized by the dilatation of the vessel wall caused by inflammation and degradation of medial layer of the aorta, followed by aortic wall rupture and bleeding, often fatal for the patient. Smoking, age, male gender and atherosclerosis are AAA risk factors, however the exact mechanisms of AAA development remain unknown. Inflammation and its mediators, cytokines, are key components of atherosclerosis development and could also contribute to AAA.

Interleukin (IL)-27 is a member of the IL-6 cytokine superfamily, regulates function of multiple hematopoietic cell subsets and some non-hematopoietic cells. We previously demonstrated that IL-27R signaling suppresses the inflammation in atherosclerosis. However, its function in AAA remains unknown.

We utilized Angiotensin II (Ang II) model to address the role of IL27R signaling in pathogenesis of AAA. Ang II containing pumps were surgically implanted into *Il27ra*$^{-/-}$ *Apoe*$^{-/-}$ or *Il27ra*$^{+/+}$ *Apoe*$^{-/-}$ control mice fed with Western Diet for 8 weeks. AAA progression was analyzed 4 weeks later. Surprisingly, we found that *Il27ra*$^{-/-}$ *Apoe*$^{-/-}$ mice were protected from AAA as apposed to controls. *Il27ra*$^{+/+}$ *Apoe*$^{-/-}$ mice developed large AAA with visual hemorrhage into the aortic wall, while small AAA in *Il27ra*$^{-/-}$ *Apoe*$^{-/-}$ mice were characterized by reduced accumulation of myeloid cells and CD4 T cells. Moreover, *Il27ra*$^{-/-}$ *Apoe*$^{-/-}$ mice had lower production of IFNγ, IL-17A, TNF-α, IL-4 and IL-13 in AAA. Downregulation of chemokines and adhesion molecules gene expression was detected in AAA of *Il27ra*$^{-/-}$ *Apoe*$^{-/-}$ mice. The analysis of hematopoietic compartment revealed strong reduction of hematopoietic stem cells (HSCs), including myeloid progenitors in bone marrow and spleen of *Il27ra*$^{-/-}$ *Apoe*$^{-/-}$ mice with Ang II pumps. Thus, IL-27R promotes the expansion of myeloid progenitor cells and their mobilization into the spleen and AAA in response to Ang II infusion during AAA formation.

PAK1 INHIBITOR FRAX1036 SENSITIZES OVARIAN CANCER CELLS WITH AMPLIFIED 11Q13 TO CYTOTOXIC EFFECT OF ROTTLERIN

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p21-activated kinases (PAKs) are Cdc42/Rac–activated serine-threonine protein kinases that regulate several key cancer-relevant signaling pathways, such as Mek/Erk, PI3K/Akt, and Wnt/b-catenin. Pak1 is frequently overexpressed and/or hyperactivated in different human cancers, including human breast, ovary, prostate, and brain cancer, due to amplification of the *PAK1* gene within an 11q13 amplicon. In previous *in vitro* and *in vivo* studies we have shown that ovarian cancer cells with amplified/overexpressed Pak1 were significantly more sensitive to pharmacologic or genetic inhibition of Pak1 than were cells without 11q13 amplification. In the present study we examined the combination effect of the Pak1 inhibitor FRAX1036 in ovarian cancer cells using ICCB Known Bioactives Library (Enzo). We found that the cytotoxic effect of FRAX1036 was significantly higher when combined with the PKC delta inhibitor, Rottlerin.

We tested FRAX1036 alone and in combination with Rottlerin on 11q13-amplified human ovarian cancer cell lines with respect to cell proliferation, migration and apoptosis. FRAX1036 alone inhibited cell proliferation and migration in 11q13-amplified ovarian cancer cell lines. Co-administration of FRAX1036 with Rottlerin further synergized the inhibition of cell proliferation *in vitro* and decreased tumor growth *in vivo*. To explain the synergistic inhibition of cell proliferation induced by the FRAX1036/Rottlerin combination we analyzed Pak1 and PKC delta down-stream signaling molecules. Western blot data indicated that FRAX1036 and Rottlerin synergistically inhibited phosphorylation of critical signaling molecules such as MEK, ERK1/2, b-Catenin, and 4EBP1.

Our findings suggest that Pak1 small molecule inhibitors in combination with Rottlerin hold potential clinical value as chemotherapeutic drugs for the ~25% of ovarian cancers characterized by *PAK1* gene amplification.

INTEGRATIVE GENOMICS ANALYSIS SUPPORTS THE ASSOCIATION OF GENETIC ANCESTRY WITH SURVIVAL DISPARITY IN HNSCC

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African-Americans (Afr-Amr) with HNSCC have a lower survival rate than Caucasians. Genome-wide analysis of ancestry informative SNPs, RNAseq, methylation, and copy number variation data for 316 oral cavity and laryngeal cancer patients resulted in the identification of five ancestry-related SNPs as cis-eQTLs (FDR<0.01) for the POLB gene. Homozygous and heterozygous genotypes containing the African (Afr) allele showed higher POLB expression relative to the homozygous Caucasian (Cau) allele genotype, with Afr-Amr patients having a significantly higher level of POLB expression compared to Caucasians (P=0.0007). The study was replicated using a GEO dataset validating all five eQTLs as well as showing a statistically significant difference in POLB expression by genetic ancestry (P=0.002). An association was observed between eQTLs and overall survival (P<0.037) as well as disease-free survival of oral cavity and laryngeal cancer patients treated with platinum-based chemo and/or radiation therapy (P=0.018 to 0.0629; N=157). Genotypes containing the Afr allele were associated with poor overall/disease-free survival compared to homozygous genotypes harboring the Cau allele. Thus, our integrative genomics analyses support the association of genetic ancestry with survival disparity in patients diagnosed with oral cavity and laryngeal cancer.

NETRIN G1 IN DESMOPLASTIC FIBROBLASTS ENHANCES INTERACTIONS WITH PANCREATIC CANCER CELLS

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Pancreatic Ductal Adenocarcinoma (PDAC) is amongst the most lethal forms of cancer encountered in patients. One of the defining characteristics of this neoplasia is a large desmoplastic stromal component that includes a highly organized fibrous extracellular matrix (ECM). Previously our lab compared ECM producing normal fibroblasts to tumor associated fibroblasts (TAFs) via mRNA microarray and noticed an interesting increase in the expression of a splice variant isoform of the synapse stabilizer Netrin G1 (NTNG1). Utilizing an *in vitro* three dimensional culturing system, produced by patient derived TAFs, we first validated isoform expression in TAFs by western blot, immunofluorescence (*in vitro* and *in vivo*) as well as via Reverse Transcriptase Polymerase Chain Reaction. We next examined the isoform expression of NTNG1 in the presence of either fibroblastic activator Transforming Growth Factor Beta (TGF-β) or its inhibitor. Our results determined that NTNG1 overexpression (compared to normal) is independent of TGF-β functions such as ECM parallel fiber orientation. The corresponding tumoral receptor Netrin G1 Ligand 1 (NGL1) was also found to be highly expressed in transformed PDAC cells. CRISPR/CAS9 knockouts of NTNG1 in TAFs and of NGL1 in PDACs were used for functional analyses. PDAC spheroid spread on three dimensional TAF cultures and live imaging of co-cultures of WT and/or knockout PDACs and TAFs demonstrated that ligand-receptor expressions are necessary for stable tumoral-stromal interactions. We speculate this function to be important for stroma supported pancreatic tumor growth, whereby stroma provides nutritional tumoral support (i.e., in the absence of angiogenesis). In conclusion, we believe disruption of this interaction has further implications in pro-tumorigenic stromal regulation.

THE ROLE OF IMPDH POLYMERIZATION IN DROSOPHILA OOGENESIS

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One of the key characteristics of cancer is increased cellular proliferation. Proliferating cells use nucleotides at an increased rate and therefore nucleotide biosynthesis is important for continued cellular proliferation and growth of cancer cells. CTP synthase (CTPS) and inosine monophosphate dehydrogenase (IMPDH) are two rate-limited enzymes in nucleotide biosynthesis, which have been found to polymerize into filaments under conditions of nucleotide depletion or elevated demand for nucleotides. For example, these enzymes polymerize in nutrient-starved mammalian cells lines or during normal Drosophila oogenesis, where germ cells undergo rapid cycles of endoreplication and rRNA synthesis. Our lab has demonstrated that these assemblies are important for Drosophila egg development and has pioneered the use of this model system to understand their function and regulation. Our hypothesis is that filament assembly enhances their nucleotide biosynthesis activity and is necessary to maintain adequate nucleotide levels in growing and proliferating cells. In order to test how and why these filaments are assembled under normal biological conditions I am utilizing mutants of the single Drosophila IMPDH gene as well as functional rescue with transgenic expression of human IMPDH2 constructs to assess the role of IMPDH filament assembly in oogenesis. I will present progress toward generating transgenic Drosophila expressing point mutants of human IMPDH2 that either inhibit or promote filament assembly without abolishing enzyme activity. This will allow me to assess the role of assembly in a biologically important in vivo context. In parallel we are evaluating the effect of these mutants on assembly and catalytic activity in vitro. These experiments will provide insight into the biological function of IMPDH filaments in vivo in a cell type undergoing rapid cycles of genomic replication. Thus, it may provide insights, and possibly new points of therapeutic intervention, into a pathway important for the proliferation and growth of cancer cells.

ANTICANCER ACTIVITY OF SYNTHESIZED PRODIGIOSIN ANALOGS

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We recently reported that Prodigiosin is a potent p53 pathway restoring small molecule that acts through p73 and by interfering with p73:mutant p53 protein interaction. Prodigiosin is the parent member of the tripyrrole alkaloid family of natural products that shows potent anti-cancer activity against tumors with mutated p53. To improve pharmacological and medicinal properties of Prodigiosin including p73 induction and restoration of the p53 pathway in tumors with mutated p53, two new series of analogs were synthesized. A number of new analogs have been prepared and tested for their anticancer activity. Preliminary biological activity assay showed that new compounds inhibited cancer cell proliferation from 0.16 μM to 0.26 μM both in p53 mutated SW480, DLD1 and wild-type HCT116 colorectal cancer cell lines. Some of the newly synthesized compounds can induce p53 transcriptional reporter activity in p53 mutated SW480 colorectal cancer cells. Our ongoing experiments are focused on the signaling mechanism of apoptosis induced by the new analogs, identification of molecular targets of the compounds, and *in vivo* studies of Prodigiosin analogues as single agents or in combination with chemotherapy or targeted therapy.

ONC201 EXERTS A ANTI-METASTATIC EFFECT AND PROMOTES AN ACCUMULATION OF NK-CELLS IN TUMOR BEARING MICE AT INCREASED DOSE AND FREQUENCIES

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ONC201, a novel first-in-class orally active anti-tumor agent that upregulates the cytotoxic ligand TRAIL and activates the integrated stress response leading to tumor cell upregulation of DR5. Currently under development by Oncoceutics, ONC201 is being evaluated in multiple phase I/II clinical trials, and has shown to be safe, with a predicted pharmacokinetics, and a preliminary efficacy signal. In the first-in human trial, patients were dosed on an every 3-week schedule. We investigated dose-intensification of ONC201 to determine whether a higher dose and frequency schedule could impact efficacy while exhibiting limited toxicity. Due to its ability to upregulate TRAIL, we hypothesized that ONC201 would inhibit metastasis and promote NK-accumulation.

We observed that ONC201 exerts a dose intensified effect on tumor progression *in vivo* and more potently suppressed Akt/ERK in tumors in a dose- and frequency-dependent manner, while effecting TRAIL serum levels solely by frequency. We noted a potent anti-metastatic effect of ONC201 which has not previously been reported, and ONC201 was seen in culture to negatively impact cancer cell migration and invasion even in ONC201-apoptotoic resistant cells. We have observed accumulation of CD3+/NK1+ cells within ONC201-treated tumors in athymic nude mice, and an upregulation of NK1+ cells in C57/BL6 and Balb/c mice and their xenografts. This accumulation within ONC201-treated tumors appeared more pronounced with dose intensification.

The results of these studies prompted a Phase II clinical trial at Fox Chase Cancer Center that is now enrolling. ONC201's anti-metastatic effects make it a promising anti-tumor agent for aggressive cancer. We are further evaluating the characteristics of the CD3+/NK1.1+ cells and the relationship of their intra-tumoral accumulation to the observed anti-tumor effects of ONC201.

STRUCTURAL AND FUNCTIONAL ANALYSIS OF A TALIN TRIPLE DOMAIN MODULE SUGGESTS AN ALTERNATIVE TALIN AUTOINHIBITORY CONFIGURATION

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Talin plays important role in regulating integrin-mediated signaling. Talin function is autoinhibited by intramolecular interactions between the integrin-binding F3 domain and the R9 domain. We determined the crystal structure of a triple domain fragment R7R8R9, which contains the autoinhibitory domain (R9) and the RIAM (Rap1-Interacting-Adaptor-Molecule)-binding domain (R8). The structure reveals a crystallographic contact between R9 and a symmetrically related R8 domain, representing an intermolecular interaction in the compact talin dimer. Strikingly, we demonstrated that the α5 helix of R9 also interacts with the F3 domain despite no contact through the α5 helix in the crystal structure of an F2F3:R9 autoinhibitory complex reported previously. Mutations on the α5 helix significantly diminish the R9:F3 association and lead to elevated talin activity. Our results offer the biochemical and functional evidence of the existence of a new talin autoinhibitory configuration, thus providing a more comprehensive understanding of talin autoinhibition, regulation, and quaternary structure assembly.

CDK4/6 INHIBITOR (PALBOCICLIB) PROMOTES CELL DEATH AND SYNERGIZES WITH IRINOTECAN IN COLORECTAL CANCER UNDER HYPOXIA IN VITRO

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We recently reported that a family of nucleoside (sangivamycin-like molecules) can sensitize tumor cells to TRAIL through dual blockade of CDK1 and GSK3b (Mayes et al., Cancer Research, 2011). We further reported that CDK inhibitors can destabilize HIF1a regardless of VHL or p53 status or the presence of hypoxia (Warfel et al., Cell Cycle, 2013). In order to translate this knowledge into a cancer therapeutic strategy, we investigated the effects of CDK inhibition in colorectal cancer (CRC) cell lines with or without chemotherapy. Palbociclib is a specific inhibitor of CDK4/6 that has been tested in numerous clinical trials for breast cancer, NSCLC, GBM, lymphoma, leukemia, in combination with 5-FU and oxaliplatin in solid malignancies (NCT01522989) or with cetuximab in head and neck cancer (NCT02101034). Little is known about the effects of CDK4/6 inhibition in CRC. We found that Palbociclib can significantly promote cell death of CRC under hypoxia. However, it is deprived of the proliferative inhibition in CRC under hypoxia, instead of normoxia where it inhibits cell proliferation via the pRb pathway. These results suggested that CDK4/6 inhibitor could distinctly function in CRC via different molecular mechanisms under hypoxia. Our data showed that Palbociclib can upregulate ERK1/2 and downregulate GSK3b signaling with different time-dependent patterns under hypoxia versus normoxia. We further found that Palbociclib synergizes with CPT-11 against CRC cell lines with different genetic subtypes. Based on our findings that Palbociclib can promote cell death of CRC under hypoxia and synergize with CPT11 *in vitro*, further investigation is needed to assess the novel combination therapy against CRC

ENHANCED HIF1A INHIBITION THROUGH DUAL INHIBITION OF CDK AND HSP90

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A prevalent characteristic of solid tumors is the presence of hypoxic areas. Intratumoral hypoxia plays a well-known role in chemo-/radio-therapy resistance and is associated with poor prognosis as well as enhanced metastasis. Hypoxia-inducible factor 1α (HIF1α) is a major mediator of the cellular response to hypoxia, which promotes malignant proliferation and progression in cancers. HIF-1α expression is increased in a variety of tumors but this is not restricted to hypoxic regions. We have previously shown that cyclin-dependent kinase 1 (CDK1) stabilizes HIF1α through direct phosphorylation of its Ser668 residue in a Von Hippel-Lindau (VHL)-independent manner both under hypoxia and at G2/M under normoxia. Another previously acknowledged VHL-independent HIF1α stabilizer is the heat shock protein 90 (HSP90) that has been correlated with adverse prognosis and recognized as a therapeutic target in cancer. We investigated potential crosstalk between CDK1-mediated and HSP90-mediated HIF1α stabilization and the potential for therapeutic targeting. Under hypoxia, the interaction between HSP90 and HIF1α proteins is impaired by CDK1 inhibition in HCT116 colon cancer cells. Heat shock treatment increases HIF1α protein level, which can be reversed by the HSP90 inhibitor, geldanamycin or the CDK1 inhibitor, Ro-3306. Moreover HIF1α level is decreased by HSP90 inhibition under hypoxia, which can be further reduced by the combination of HSP90 inhibition and CDK1 knockdown. Combinational inhibition of CDK1 and HSP90 synergistically suppresses HCT116 proliferation under both normoxia and hypoxia. The joint inhibitory effect of CDK1 and HSP90 on HIF1α level is observed in other colorectal cancer cell lines as well.

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