Rare Tumors Initiative Symposium

Strategies to develop therapies for rare tumors: Small numbers, but big opportunities

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Functional genomic screening reveals splicing of the EWS-FLI1 fusion transcript as a vulnerability in Ewing sarcoma - Dr. Natasha Caplen

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Ewing sarcoma (ES) is a highly aggressive cancer of the bone and soft tissue. In ~85% of ES tumors the primary oncogenic event is a t(11:22)(q24;q12) translocation that generates a fusion of the 5' end of EWSR1 and the 3' end of FLI1 referred to as EWS-FLI1. The exact genomic breakpoints within the EWSR1 and FLI1 genes vary, but typically occur within introns and require the splicing machinery to generate an in-frame EWS-FLI1 transcript. The most common EWS-FLI1 transcripts fuse either exon 7 of EWSR1 to exon 6 of FLI1 (a type I or a 7/6 fusion), or fuse exon 7 of EWSR1 to exon 5 of FLI (a type II or 7/5 fusion). In an estimated 40% of EWS-FLI1 driven tumors the generation of an in-frame EWS-FLI1 fusion transcript requires alternative splicing. In particular, translocations that retain exon 8 of EWSR1 generate an out-of-frame transcript unless this exon is removed. Using an assay of EWS-FLI1 activity and genome-wide siRNA screening we have identified RNA processing as a therapeutic vulnerability in ES. Parallel genome-wide siRNA-mediated RNAi screens were conducted in ES TC32 cell lines expressing a luciferase (luc) reporter protein driven by either the promoter of the EWS-FLI1 target gene NR0B1 (TC32-NR0B1-luc) or the CMV promoter (TC32-CMV-luc). The top gene ontology terms associated with the 28 priority candidate genes that when silenced induced a differential decrease in the TC32-NR0B1-luc signal versus the TC32-CMV-luc signal were mRNA splicing (p-value = 1.42E-08) and mRNA processing (p-value = 2.32E-07). To investigate the mechanistic basis for the identification of specific RNA processing proteins as required for the activity of EWS-FLI1 we focused our analysis on two proteins involved in pre-mRNA splicing. Using PCR analysis we have determine that EWS-FLI1 fusion transcripts are sensitive to the loss-of-function of specific splicing factors and using RT-PCR, immunoblot, and whole transcriptome analysis have shows that disrupted splicing of the EWS-FLI1 transcript alters its expression and reverses the expression of a significant proportion of genes that are targets of EWS-FLI1. Our results provide the basis for a novel strategy to target fusion oncogenes by interfering with RNA processing. This study has implications for the treatment of ES through inhibition of proteins required for expression of the EWS-FLI1 transcript. Our findings may also open up strategies for treatment of other cancers driven by fusion oncogenes.

This research was supported by the Intramural Research Program of the National Cancer Institute, Center for Cancer Research NIH, DHHS and by funding to P.J.G. from the Lily's Garden Foundation, the Sarcoma Alliance for Research Through Collaboration, and the Turner-Hazinski Award, Vanderbilt University. We thank Zhili Zheng (Surgery Branch, CCR, NCI), Amy McCalla (Pediatric Oncology Branch, CCR, NCI), and Thorkell Andersson (Protein Characterization Lab, Cancer Research Technology Program at NCI-Frederick, NCI) for technical assistance and Javed Khan for SKNMC cells. We also thank members of the Genetics Branch, CCR, NCI, in particular, Javed Khan, Ashish Lal, Shile Zhang, John Shern, Young Song, Sean Davies, and Josh Waterfall for helpful discussion.
Multiple dermatofibrosarcoma protuberans (DFSP) in patients with adenosine deaminase deficiency-associated severe combined immunodeficiency (ADA-SCID) - Dr. Edward Cowen

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DFSP is a rare skin tumor with characteristic cytogenetic findings (t(17;22)(q22;q13)) resulting in a COL1A1-PDGFB fusion gene. In this study, 11 consecutive patients with ADA-SCID were examined at the NIH Clinical Center. Skin biopsies of suspicious lesions were performed, followed by H&E and staining for CD34 and Factor XIIIa. DFSP was identified in 9/11 patients (age: 1-22 years). Multiple lesions (range 2-14) were identified in 7/9 patients. Most lesions were small tan/brown indurated oval plaques consistent with early DFSP. All lesions showed a spindle cell proliferation in the dermis, extending into the subcutaneous fat. A storiform histologic pattern was detected only in the adult patient. In all cases, CD34 expression was diffusely positive and FXIIIa was negative. FISH was positive for COL1A1-PDGFB in 4/6 patients studied. RT-PCR showed a COL1A1-PDGFB fusion transcript in one additional case. We describe a high prevalence of DFSP in the ADA-SCID population. Two cases of ADA-SCID-associated DFSP have been described, however this is the first report of multicentric lesions. Understanding the etiology of multiple DFSP lesions in the setting of ADA-SCID may provide eventual insight into the pathogenesis of this rare sarcoma.
Drug Combination Screening for Ewing's Sarcoma - Dr. Mindy Davis

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Ewing's sarcoma is a rare bone cancer that primarily affects individuals under the age of 30. Ewing's sarcoma is treated with cytotoxic drug combinations, and there is a need for new treatment options. Four Ewing's cell lines were screened against the 1912 small molecule compounds in the MIPE (mechanism interrogation plate) library. Inhibitors of cell viability were identified. Combinations of identified inhibitors were then screened together in a matrix format to look for synergistic and additive combinations. The screen identified several candidate combinations that exhibited synergistic behavior at multiple dose combinations and we report on follow up studies exploring these synergies in more detail.

This work was supported by the Division of Preclinical Innovation, National Center for Advancing Translational Sciences and Center for Cancer Research, National Cancer Institute.
A comparison of RECIST and semi-automatic volumetric measurements as predictors of clinical outcome in chordoma patients receiving immunotherapy - Ms. Kathleen Fenerty

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PURPOSE
The Response Evaluation Criteria in Solid Tumors (RECIST) is the current standard for assessing therapy response in patients with malignant solid tumors. There has been concern among oncologists that RECIST lacks sensitivity to detect growth or impact of therapeutic agents; this is of particular interest in chordoma due to relatively slow growth. Technological advances such as semi-, and potentially, fully-automated segmentation methods have recently allowed volumetric segmentations to be feasible within Picture Archiving and Communication Systems (PACS). We aim to evaluate the usefulness of volumetric measurement as a predictor of clinical outcome in chordoma patients and to assess the feasibility of collecting volumetric measurements from each patient.

METHODS AND MATERIALS
Manual RECIST measurements were acquired for 21 chordoma patients participating in two IRB-approved studies. Using semi-automatic segmentation tools within our PACS (v12.0, Carestream, Rochester, NY), lesions were segmented and volumes were calculated for every tumor at baseline and post-treatment. Magnetic Resonance (MR) was used for primary tumors and Computed Tomography (CT) was used for metastatic lesions. The volumetric measurements were plotted against RECIST progression, progression-free survival, overall survival, and subjective pain measurement.

RESULTS
Our pilot study has thus far found segmentation of each tumor to be feasible within PACS in 21 chordoma patients. Volumetric segmentation of tumors for comparison with clinical outcomes is ongoing. Data collection concerning accuracy of semi-automatic segmentation is also ongoing and will be presented at a later date. Here, we present our segmentation methodology and rationale with representative images of volumetric analysis for illustrative purposes.

CONCLUSION
In the coming weeks, we plan to have enough data to present a preliminary comparison between RECIST and volumetric measurements.

CLINICAL RELEVANCE/APPLICATION
Semi-automatic volumetric measurement of tumors should allow for more accurate prognoses and improved assessment of cancer treatment.
Changes in Cholesterol Metabolism Support High Density Cell Growth and Therapeutic Resistance in Glioblastoma Multiforme - Dr. Diane Kambach

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Glioblastoma Multiforme (GBM) is a highly aggressive, therapeutically resistant brain tumor that arises from cells of astrocytic lineage. The five year survival rate is just below 10% and this statistic has changed very little in the past decade. Amplification and activating mutations of receptor tyrosine kinases (RTKs) are common in GBM; however despite their oncogenic importance, RTK inhibition with tyrosine kinase inhibitors (TKIs) has been clinically ineffective for GBM. The gap between promising laboratory results and clinical success is a common obstacle in cancer research in part because cell culture models do not accurately mimic complex in vivo conditions and cell behavior, such as therapeutic resistance. Many cell culture studies and high throughput screens of pharmacological agents are performed in cells plated at sub-confluence, or low density. We have found that GBM tumor initiating cells (GBM-TICs) are sensitive to TKIs at low density; however as culture density increases they become resistant, despite equal inhibition of the target RTK and downstream signals. Thus, culturing cells at low and high density provides a novel, isogenic system for studying the cellular mechanisms of therapeutic response. While density does not affect the cell cycle profile of GBM-TICs, we have found that high density cells have lower levels of reactive oxygen species and ATP than low density cells and reduced mitochondrial membrane potential. This suggests a switch to aerobic glycolysis (the Warburg Effect); however lactate levels, which should increase under conditions of aerobic glycolysis, are unaffected by cell density. Additionally, assessment of extracellular metabolic flux does not support an increase in aerobic glycolysis in dense GBM-TICs. Extracellular acidification rate (ECAR), which should increase with aerobic glycolysis, does not show a tumor-specific effect. Oxygen consumption rate (OCR), which should decrease during aerobic glycolysis, increases significantly with density in GBM-TICs but shows no density-dependent change in healthy astrocytes. These data indicate a tumor-specific, non-Warburg metabolic shift in dense GBM-TICs that is not present in sparse GBM-TICs or normal human astrocytes at any density. Further analysis has implicated changes in cholesterol metabolism and transport in density-dependent metabolic change. Specifically, densely cultured GBM-TICs have higher levels of cholesterol and elevated expression of cholesterol transport proteins such as ABCA1 than cells cultured sparsely. Pharmacologic inhibition of cholesterol biosynthesis results in reduced OCR and increased ATP per cell in dense but not sparse GBM-TICs, which corroborates the importance of cholesterol synthesis and metabolism in dense GBM-TICs. We are currently investigating the connection between cholesterol metabolism and therapeutic resistance in GBM in the hope of identifying novel therapeutic targets to improve clinical outcomes for GBM patients.
Treatment of Ocular Melanoma in a Rabbit Orthotopic Model Using a Novel Tumor-tropic Viral-like Nanoparticle Dye Conjugate - Ms. Rhonda Kines

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Ocular melanoma (OM), while the most common tumor arising in the eye, is considered to be a rare cancer with around 2,000-2,500 new cases diagnosed annually in the United States. This disease is highly metastatic (liver) and the usual treatment for small tumors is brachytherapy, which can lead to vision loss in >70% of patients, whereas larger tumors require enucleation. Neither of these treatment methods has been shown to reduce the risk of metastasis, highlighting the importance of developing a treatment modality that could be applied to tumors at an earlier stage. We have found that human papillomavirus (HPV) virus-like particles (hereafter revered to as viral-like nanoparticles) display a natural tropism for tumors, likely through cell surface interactions with specifically modified forms of heparan-sulfate proteoglycans (HSPG) that mimic the specific HSPGs mediating normal HPV infection of disrupted epithelia. Researchers at the NCI have demonstrated that the near infrared (nIR) dye, 700DX, when conjugated to antibodies and bound to tumor cells, leads to immediate cellular cytotoxicity upon excitement with nIR light. We therefore have conjugated 700DX to the viral-like nanoparticles for the purposes of targeting OM using a rabbit model in which human OM cells, 92.1, are implanted in the choroid of immune suppressed rabbits. In a pilot study rabbits were divided into groups based on the size of their tumors (large >4mm, medium 2mm-4mm, small <2mm) and all rabbits received three weekly treatments consisting of intravitreal injections of the viral-like nanoparticle conjugate followed by tumor exposure to nIR light 16hr later. Two rabbits remained as untreated controls, and nine were treated. 5/9 animals displayed complete regression (2/5 large, 1/2 medium and 2/2 small) and 4/9 had tumor responses as defined by growth arrest or tumor shrinkage (3/5 large, 1/2 medium). Tumor shrinkage was observed within one week of the first treatment in nearly all animals, and upon pathological inspection three weeks after the third and final treatment, no tumor could be observed in the complete responders and >50% tumor necrosis was noted in the other treated animals. Additionally, no damage to the retina was observed after treatment. Larger scale dose/response studies are currently on-going and to date, these studies are recapitulating the complete responses and tumor shrinkage previously observed. Overall, the viral-like nanoparticle dye conjugate is a promising first line treatment for patients diagnosed with OM, with the potential to effectively treat their tumors while sparing their vision.
CDCA7L cooperates with KDM5D to promote gliomagenesis specifically in males
Dr. Min-Hyung Lee

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The most common types of primary brain tumors, astrocytoma and glioblastoma multiforme (GBM), are currently incurable. Both astrocytoma and GBM show male predominance, with a male to female ratio of 1.42:1 and 1.58:1, respectively. We performed linkage analysis in the Nf1-/+;Trp53-/+cis (NPCis) mouse model of astrocytoma/GBM to identify a male-specific gliomagenesis modifier and further used combinatorial bioinformatics approaches as well as cross-species comparisons to prioritize male-specific candidate gene(s). Here, we showed that Cell division cycle-associated 7-like (CDCA7L), a myc co-transcription factor, is a male-specific oncogene in astrocytoma/GBM. CDCA7L expression is up-regulated in astrocytoma cells compared with normal brain, with males showing higher levels than females in both human and mouse. shRNA-mediated CDCA7L knockdown led to the decreased cell growth and viability of male-derived astrocytoma cells, but not female tumor cells in both human and mouse. Further mechanistic studies showed that CDCA7L depletion in male-derived astrocytoma cells led to the induction of cleaved Caspase-3 and p27 expression and reduction of Cyclin D1 expression. Furthermore, Cdc7l overexpression promotes the growth of WT mouse primary astrocytes only in males by inducing Cyclin D1 expression, but not in female astrocytes, suggesting a male-specific oncogenic role for CDCA7L in astrocytoma/GBM. Strikingly, CDCA7L depletion in human female U87MG GBM cells caused the increase of both growth and viability opposite to what is seen in male cells. Because male-female differences in CDCA7L action are hormone-independent, we examined whether the male-specific histone demethylase KDM5D regulates the effects of CDCA7L. We found that knockdown of KDM5D inhibits male-specific effects of CDCA7L on p27 and Cyclin D1 expression. Our data highlight the sex-specificity of CDCA7L in astrocytoma/GBM tumorigenesis and show that CDCA7L signaling pathways can be oncogenic in males, while being neutral or tumor suppressive in females. This has important implications for the application of therapies to human GBM patients.

This research was supported by the Intramural Research Program of the NIH, NCI, and with Federal funds from the National Cancer Institute under contract N01-CO-12400 to Leidos-Frederick. We thank Dr. Toshiyuki Sakai for providing reagents, and Drs. Xiaolin Wu, David Sun, and Ling Su of the Laboratory for Molecular Technology Core for assistance with microarray experiments.
CDK4 amplification reduces sensitivity to CDK4/6 inhibition in fusion-positive rhabdomyosarcoma - Dr. Mary Olanich

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Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma and includes an aggressive, PAX3-FOXO1 fusion-positive subtype. Amplification of chromosomal region 12q13-q14, which contains the CDK4 proto-oncogene, was identified in a subset of fusion-positive RMS. Other tumor types with CDK4 amplification or overexpression, including liposarcoma and neuroblastoma, are sensitive to CDK4/6 inhibition, suggesting that CDK4/6-targeted therapies may provide a new treatment strategy in fusion-positive RMS. To evaluate the role of CDK4 in chromosomal region 12q13-14 amplification in fusion-positive RMS and the potential clinical utility of CDK4/6 inhibition in this disease setting, we examined the biological consequences of CDK4 knockdown, CDK4 overexpression, and pharmacologic CDK4/6 inhibition in fusion-positive RMS in vitro and in vivo. Knockdown of CDK4 abrogated proliferation and transformation of 12q13-14-amplified and non-amplified fusion-positive RMS cells via G1-phase cell cycle arrest. This arrest was associated with reduced RB phosphorylation and E2F-responsive gene expression. Significant differences in E2F target expression, however, were not observed in RMS cells overexpressing CDK4 or in fusion-positive tumors harboring 12q13-14 amplification relative to control cells or tumors lacking amplification, respectively. Treatment with the small molecule CDK4/6 inhibitor LEE011 phenocopied CDK4 knockdown, decreasing viability, RB phosphorylation, and E2F-responsive gene expression and inducing G1-phase cell cycle arrest. All fusion-positive RMS cell lines showed sensitivity to CDK4/6 inhibition, with evidence of differential antitumor activity resulting from an inverse relationship between CDK4 expression and inhibitor vulnerability. This variable responsiveness to LEE011 was recapitulated in xenograft models of CDK4-amplified and non-amplified fusion-positive RMS. Our findings demonstrate that CDK4 is necessary for RB-E2F-mediated G1-phase cell cycle progression, proliferation, and transformation in fusion-positive RMS regardless of CDK4 amplification status. Our studies further indicate that single-agent LEE011 is active in the setting of fusion-positive RMS and suggest that CDK4 amplification may be a marker of reduced sensitivity whereas low CDK4 expression may be associated with higher susceptibility to CDK4/6 inhibition. Collectively, our data provide preclinical evidence supporting further investigation of CDK4/6-targeted therapies in treatment regimens for fusion-positive RMS.
**PAX3-FOXO1 is essential for initiation but not for recurrence during rhabdomyosarcoma tumorigenesis.** - Dr. Puspa Pandey

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The PAX3-FOXO1 fusion gene, which is generated by a 2; 13 chromosomal translocation, is a characteristic feature of fusion-positive rhabdomyosarcoma (RMS), a major RMS subtype associated with aggressive behavior and poor prognosis. This study utilizes a novel inducible expression system in human myoblasts to dissect the molecular mechanism and contribution of PAX3-FOXO1 in RMS tumorigenesis. A human myoblast cell line (immortalized by BMI1 and TERT expression) was transduced with a retroviral construct that constitutively expresses MYCN and a lentiviral based- doxycycline inducible construct that variably and reversibly expresses PAX3-FOXO1. Focus formation and animal xenograft experiments were performed to study transformation in vitro and tumorigenesis in vivo, respectively. Myogenic differentiation was assessed by light microscopy and by western blot or immunohistochemical assays of muscle-specific protein expression. PAX3-FOXO1-transduced myoblasts treated with doxycycline demonstrated a time- and dose-dependent increase in expression of PAX3-FOXO1 mRNA and protein and its downstream targets genes; doxycycline withdrawal led to cessation of fusion protein expression. Though myoblasts expressing PAX3-FOXO1 or MYCN alone did not show any evidence of transformation in culture, combined PAX3-FOXO1 and MYCN expression resulted in myoblast transformation. Under differentiation-promoting culture conditions, combined PAX3-FOXO1 and MYCN expression inhibited myogenic differentiation. Intramuscular injection of myoblasts with MYCN and PAX3-FOXO1 resulted in rapid RMS tumor formation in NOD-SCID mice when fusion protein expression was induced by feeding mice a doxycycline-containing diet. Myoblasts with MYCN expression alone did not form any tumors while PAX3-FOXO1 induction without MYCN expression resulted in RMS tumors only after a much longer latency period. After tumors formed from myoblasts expressing PAX3-FOXO1 with or without MYCN, down-regulation of PAX3-FOXO1 expression by doxycycline withdrawal resulted in tumor regression associated with widespread myogenic differentiation. However, the regressed tumors slowly grew back in the absence of doxycycline induction demonstrating a PAX3-FOXO1-independent oncogenic mechanism for recurrence. The PAX3-FOXO1 fusion protein collaborated with MYCN in the initial stage of RMS tumorigenesis to promote dysregulated cell proliferation and inhibit myogenic differentiation. Though most cells in the initial tumor were dependent on the fusion protein, recurrent tumors formed in which the fusion protein was not required to maintain the tumorigenic phenotype.
Targeting ABL1-Mediated Oxidative Stress Adaptation in Fumarate Hydratase-Deficient Cancer - Dr. Carole Sourbier

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Patients with germline fumarate hydratase (FH) mutation are predisposed to develop aggressive kidney cancer with few treatment options and poor therapeutic outcomes. An unbiased drug screen using FH-deficient tumor-derived cell lines identified the tyrosine kinase inhibitor vandetanib to be highly cytotoxic both in vitro and in vivo. Mechanistic studies revealed that vandetanib-mediated cytotoxicity is ABL1-dependent. We discovered that the activity of the proto-oncogene ABL1 is upregulated in FH-deficient kidney tumors and drives a metabolic and survival signaling network necessary to cope with impaired mitochondrial function and abnormal accumulation of intracellular fumarate. Excess fumarate indirectly stimulates ABL1 activity while restoration of wild-type FH abrogates both ABL1 activation and the cytotoxicity caused by ABL1 inhibition or knockdown. ABL1 upregulates aerobic glycolysis via the mTOR/HIF1α pathway and neutralizes fumarate-induced proteotoxic stress by promoting nuclear localization of the antioxidant response transcription factor NRF2. In animal xenograft studies, an 8-week regimen of vandetanib and metformin treatment resulted in 13 months of tumor-free survival. Thus, inhibiting ABL1 may provide a clinically feasible strategy for treating patients with highly aggressive FH-deficient kidney cancer and perhaps additional glycolytic, oxidatively stressed tumors.
Drug repurposing screen to identify compounds that resensitize cisplatin in resistant ovarian cancer - Dr. Wei Sun

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Ovarian cancer is the most lethal malignancy of the female reproductive system and the fifth leading cause of cancer death in women. Unfortunately, the types of chemotherapeutical agents for treating ovarian cancers are rather limited. Currently, platinum-based chemotherapy is the primary treatment for ovarian cancer. However, acquired resistance to platinum agents during treatment impedes the success of the treatment. In the clinic, most ovarian cancers become progressively refractory to cisplatin (cis-diamminedichloroplatinum II, CDDP) over time, resulting in unfavorable prognosis. Thus, there is an urgent need to develop novel anti-ovarian cancer agents and agents that can augment and increase the efficacy of currently available drugs. We developed a cisplatin-resistant ovarian cancer model by chronic exposure of the cisplatin-sensitive cell line to increasing concentrations of cisplatin. This cisplatin-resistant ovarian cancer is over 20-fold resistant to cisplatin than sensitive ovarian cancer. Using this cancer model, an ATPlite viability assay was developed for high throughput screening. A total of 5000 compounds that are either FDA approved drugs or used for clinical investigations were tested in this assay in the presence of low concentration of cisplatin. A total of 60 drugs were identified and confirmed with EC50 values = 1 M against cisplatin-resistant ovarian cancer. Three of them showed strong synergies with cisplatin, resensitizing cisplatin in resistant cancer model. NEDD8-activating enzyme is one of the proposed targets. Therefore, this study provides potential candidates for drug development of cisplatin-resistant ovarian cancer, as well as targets and pathways for improving our understanding of resistance.

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Distinct Methylation Profiles Characterize Fusion-Positive and Fusion-Negative Rhabdomyosarcoma - Dr. Wenyue Sun

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Rhabdomyosarcoma comprises two major subtypes, fusion-positive (PAX3-FOXO1 or PAX7-FOXO1) and fusion-negative. To investigate the significance of DNA methylation in these subtypes, we analyzed methylation profiles of 37 rhabdomyosarcoma tumors and 10 rhabdomyosarcoma cell lines as well as 8 normal tissues. Unsupervised clustering of DNA methylation clearly distinguished the fusion-positive and fusion-negative subsets. The fusion-positive tumors showed substantially lower overall levels of methylation compared to fusion-negative tumors. Comparison to the methylation pattern of normal skeletal muscle and bone marrow indicates that fusion-negative rhabdomyosarcoma is more similar to these normal tissues than fusion-positive rhabdomyosarcoma, and suggests that many of the methylation differences between these subtypes arise from aberrant hyper- and hypomethylation events in fusion-positive rhabdomyosarcoma. Integrative methylation and gene expression analysis revealed that methylation differences between fusion-positive and fusion-negative tumors could either be positively or negatively associated with mRNA expression. There was no significant difference in the distribution of PAX3-FOXO1 binding sites between genes with and without differential methylation. However, the finding that PAX3-FOXO1 binding sites were enriched among genes that were both differentially methylated and differentially expressed suggests that the fusion protein interacts with DNA methylation to regulate target gene expression. An 11-gene DNA methylation signature, classifying the rhabdomyosarcoma tumors into fusion-positive and fusion-negative subsets, was established and validated by pyrosequencing assays. Notably, EMILIN1 (part of the 11-gene signature) showed higher methylation and lower mRNA expression in fusion-positive compared fusion-negative tumors, and demonstrated demethylation and re-expression in multiple fusion-positive cell lines after treatment with 5-aza-2’-deoxycytidine. In conclusion, our study demonstrates that fusion-positive and fusion-negative rhabdomyosarcoma tumors possess characteristic methylation profiles that contribute to the expression differences between these fusion subtypes. These findings indicate an important relationship between fusion status and epigenetic changes in rhabdomyosarcoma, present a novel approach for ascertaining fusion status, and may identify new therapeutic targets in rhabdomyosarcoma.
Development of a Novel Plexiform Neurofibroma Farming System for 96-well Plate Drug Screening. - Dr. Emmanuel Tavares

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Establishing long-term primary tumor cell culture is essential in the study of rare nervous system diseases such as Neurofibromatosis type 1 (NF1). The development of novel murine models has provided significant insight into NF1 tumor biology, yet clinical outcomes have not significantly improved over the last decade. Therefore, primary cultures obtained directly from patients will be vital in designing personalized therapies. NF1 plexiform neurofibroma tumors are primarily composed of Schwann cells (SC) that are particularly slow growing and have limited passage numbers. We are focused on overcoming these limitations by targeting the interaction between SC and Extracellular Matrix (ECM) proteins that promote proliferation. From initial 2D high throughput screen (HTS), we did not observe biologically relevant improvements in SC viability and proliferation on whole protein coated plates compared with uncoated. We are testing 2-way 2D combinations of whole proteins and will transition into 3D culture systems, testing both whole proteins and ECM peptide mimetics (Amsbio). Our data suggest that optimization of ECM proteins for SC culture may aid in overcoming the limited lifespan of primary cell culture.

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Hippo pathway effector TAZ is required for drug resistance and tumorigenesis of glioblastoma stem cells. - Dr. Carlos Tristan

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Glioblastoma Multiforme (GBM) is an extremely aggressive brain cancer characterized by rapid progression, high resistance to current therapeutic regimens, and survival rates of only 25% two-years post diagnosis. These characteristics of GBM are attributed to glioblastoma stem cells (GSCs), a highly tumorigenic subpopulation of cancer stem cells that have been identified as the true therapeutic targets for the treatment of GBM. Normal organ and tissue growth during development is regulated by the Hippo pathway, wherein cell-to-cell contact-induced activation of this kinase cascade inhibits cell growth and proliferation. In recent years, studies have suggested that dysregulation of the Hippo signaling pathway might underlie cancer progression, poor overall patient survival and treatment resistance in GSCs. Since high grade gliomas are positively correlated with the expression of TAZ; a key downstream effector of the Hippo pathway, and therapeutic resistant stem cells give rise to GBM, we hypothesized that dysregulation of the Hippo pathway in cancer stem cells may play a significant role in the propagation and sustainment of GBM. To address this question, we conducted loss-of-function studies using shRNAs against TAZ in GSCs and determined if TAZ plays a functional role in drug resistance and tumorigenesis in GBM. Here we demonstrate that TAZ knockdown sensitizes GSCs to chemotherapeutic tyrosine kinase inhibitors (TKIs) via a 10-fold increase in efficacy, when compared to controls. Furthermore, we demonstrate that knockdown of TAZ abrogates several tumorigenic characteristics of GSCs, including anchorage-independent cell growth, cell migration and invasiveness. In addition, gain-of-function studies using overexpression of TAZ in immortalized normal human astrocytes (iNHAs) confers anchorage-independent cell growth and augments cell migration in iNHAs. Our studies suggest that TAZ is functionally associated with resistance to TKIs and multiple tumorigenic characteristics of glioblastoma stem cells. Furthermore, the tumorigenic properties acquired by immortalized NHAs via overexpression of TAZ suggest that TAZ might drive the transformation of normal cells into cancerous cells. An increased understanding of the molecular interactions underlying the functional role of TAZ in drug resistance and tumorigenesis will help identify potential therapeutic targets for the treatment of GBM.

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Developing therapies for rare tumors: using mouse models of malignant peripheral nerve sheath tumors to complement rare human samples in drug screens. - Mr. Robert Tuskan

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Rare cancers are defined by the U.S. Orphan Drug Act as affecting <200,000 patients in the United States. For patients with these cancers, there may be an urgent need for new therapies; however, these rare cancers present challenges to drug development. Because of the small number of patients, the cancers are not well characterized, the molecular mechanisms may not be well understood, and there may not be adequate model systems available for research and drug development. The Rare Tumors Initiative in the Intramural Research Program at the National Cancer Institute is focusing on overcoming hurdles in rare tumor research by developing new approaches to treat malignant peripheral nerve sheath tumors (MPNST). MPNSTs occur in younger adults with an incidence of 1 in 100,000, with a higher incidence in patients with Neurofibromatosis type 1. Due to a limited number of high quality human MPNST tumor lines, drug testing has been challenging both in vitro and in vivo. A genetically engineered mouse model carrying mutations in Nf1 and Trp53 develops MPNST spontaneously starting around 3 months of age. We have generated tumor lines from over 30 independent mouse MPNSTs, from different sexes and genetic backgrounds that we are using to test drugs in combination with available human MPNST lines. We find good concordance between the mouse and human MPNST lines in response to drugs. Furthermore, although the MPNST lines show good dose response and high maximum response to many targeted compounds, the concentrations of drug required to achieve inhibition is often high, suggesting that one of the difficulties in developing MPNST therapy is the inherent resistance of MPNST cells to drug inhibition.
RAS-directed therapy for fusion negative rhabdomyosarcoma - Dr. Marielle Yohe

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Background/Objectives: Fusion negative rhabdomyosarcoma (RMS) is a rare tumor affecting approximately 250 children and adolescents annually. The cure rate for relapsed or metastatic fusion negative RMS remains poor, despite aggressive multi-modality treatment associated with numerous life-threatening toxicities. Novel treatment approaches are needed to improve overall survival in relapsed or metastatic RMS. The RAS-RAF-MEK-ERK MAP kinase pathway plays a critical role in the development of many cancers. Recent comprehensive genomic analyses of human fusion negative RMS reveal that the most common somatic mutation is an oncogenic change in one of the RAS isoforms, namely NRAS, HRAS or KRAS. We hypothesize that RAS effector pathway inhibitors will have limited or transient anti-tumor efficacy but combinations of RAS effector pathway inhibitors will provide measurable clinical benefit in fusion negative RMS.

Methods: The RD (NRAS Q61H) and CTR (HRAS Q61K) RMS cell lines and the C2C12 mouse myoblast cell line were used in this analysis. Clonogenic, myogenic differentiation, cell viability, cell cycle, immunofluorescence, immunoblotting and xenograft experiments were performed according to standard protocols. Gene expression analysis was performed on RD/CTR cells treated with 10 or 100 nM trametinib for various periods of time (6, 24 or 48 hours) or treated with vehicle for 48 hours. Poly-A selected RNA libraries were prepared and sequenced on an Illumina HiSeq2000. Results: Stable knock-down of oncogenic RAS expression in RD and CTR leads to induction of cell death by apoptosis. In addition, overexpression of mutant RAS isoforms in C2C12 myoblasts inhibits myogenic differentiation induced by low-serum conditions. This differentiation block is mediated by activation of the RAF-MEK-ERK pathway. In parallel experiments, we performed an unbiased screen of the ability of small molecules to impact cell viability using a panel of 1916 molecules on RD and CTR. In this screen, the most potently selective class of molecules was the MEK1/2 inhibitors. We focused further investigation on trametinib, an allosteric, non-ATP competitive inhibitor of MEK1/2 that was recently FDA approved for the treatment of unresectable or metastatic BRAF-mutated melanoma. Trametinib treatment decreases cell proliferation, increases apoptosis, and induces G1 arrest and skeletal muscle differentiation in RAS-mutated RMS cell lines. Expression of muscle specific genes is increased and expression of genes necessary for cell cycle progression is decreased with trametinib treatment. In addition, trametinib treatment slows tumor growth and prolongs survival in fusion negative RMS xenograft models. Conclusions/Future Directions: Constitutively active RAS is necessary and sufficient for rhabdomyosarcomagenesis. The RAF-MEK-ERK pathway is activated downstream of oncogenic RAS in fusion negative RMS cell lines. Inhibition of this aberrant MEK activity with trametinib leads to skeletal muscle differentiation. Future work is aimed at uncovering the ERK-dependent transcription factors responsible for the differentiation block in fusion negative RMS and identifying mechanisms of resistance to inhibitors of RAS effector pathways in RMS.
New High Affinity Monoclonal Antibodies Recognize Non-Overlapping Epitopes On Mesothelin For Monitoring And Treating Mesothelioma - Dr. Yifan Zhang

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Mesothelin is an emerging cell surface target in mesothelioma and other solid tumors. Most antibody drug candidates recognize highly immunogenic Region I (296?390) on mesothelin. Here, we report a group of high-affinity non-Region I rabbit monoclonal antibodies. These antibodies do not compete for mesothelin binding with the immunotoxin SS1P that binds Region I of mesothelin. One pair of antibodies (YP218 and YP223) is suitable to detect soluble mesothelin in a sandwich ELISA with high sensitivity. The new assay can also be used to measure serum mesothelin concentration in mesothelioma patients, indicating its potential use for monitoring patients treated with current antibody therapies targeting Region I. The antibodies are highly specific and sensitive in immunostaining of mesothelioma. To explore their use in tumor therapy, we have generated the immunotoxins based on the Fv of these antibodies. One immunotoxin (YP218 Fv-PE38) exhibits potent anti-tumor cytotoxicity towards primary mesothelioma cell lines in vitro and an NCI-H226 xenograft tumor in mice. Furthermore, we have engineered a humanized YP218 Fv that retains full binding affinity for mesothelin-expressing cancer cells. In conclusion, with their unique binding properties, these antibodies may be promising candidates for monitoring and treating mesothelioma and other mesothelin-expressing cancers.

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