Abstracts

**TBI-17. INTEGRATIVE ANALYSES OF BRAFV600E MUTATED GLIOMAS: FROM MOLECULAR BIOLOGY TO RADIOLOGY AND TREATMENTS**

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BRAFV600e mutation is encountered mostly in low-grade pediatric gliomas (LGG) and epileptogenic glioneuronal tumors, such as gangliogliomas (GG). Less frequently this mutation is present in high-grade glial (HGG) or gliosarcomatous tumors. Recent publications were highlighting BRAF mutation and CDKN2A deletion, as independent prognostic factors linked to a worse outcome in LGGs. We studied retrospectively a monocentric cohort of 12 LGGs (9 GG and 3 pilocytic astrocytomas) and 9 HGG (5 “de novo” tumors and 4 with a long past of LGG evolution) with BRAFV600e positivity. The patients were aged from 1 to 47 years. LGGs were under 20 years and only 3 patients with HGGs had less than 18 years. We focused on extended tumors’ biology assessment by DNA single-cell analyses, RNAsequencing, NGs, metabolomics, radiology (MRI), PET-scanning and spectroscopy) and correlated them to tumor’s data. One LGG had a CDKN2A deletion. Six had a complete surgical resection, 2 had a minimal residue and 4 had chemotherapies after partial surgery and relapsed. All HGGs had a surgical resection followed by chemotherapy and radiotherapy and additional GBM. For the 2 pediatric HGGs, we identified distinct tumor clusters from single-cell analyses including drug screening. To illustrate the role of drivers in targeted therapy, we performed a functional screen in GBM-resistant cells. To further functional screening of the spheres, we mechanically dissociated the tissue and digested it in trypsin. The cells isolated were cultured in serum-free DMEM medium. Immunocytochemistry analysis was then done to compare the spheres and original GBM, the second passage. Profiling with targeted sequencing and proteomics data reveals unique biology associated with H3K27M mutation status in pediatric brain tumors including functional insight that helps drive translational efforts.

**TBI-21. LNC-TALC PROMOTES O-METHYLGUANINE-DNA METHYLTRANSFERASE EXPRESSION VIA REGULATING THE C-MET PATHWAY BY COMPETITIVELY BINDING WITH MIR-20B-3P**

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Long noncoding RNAs (lncRNAs) have emerged as new regulatory molecules implicated in diverse biological processes, including therapeutic resistance. However, the mechanisms underlying lncRNA-mediated temozolomide (TMZ) resistance in glioblastoma (GBM) remain largely unknown. To illustrate the role of lncRNA in TMZ resistance, we induce TMZ-resistant GBM cells, perform a lncRNA microarray of the parental and TMZ-resistant GBM cells, and perform a lncRNA microarray of the parental and TMZ-resistant GBM cells. Furthermore, lnc-TALC-mediated (lncRNA in glioblastoma recurrence), correlated with TMZ resistance via competitively binding miR-20B-3p to facilitate c-Met expression. A phosphorylated AKT/FoxO3 axis regulated lnc-TALC expression and c-Met in TMZ-resistant GBM cells. Furthermore, Inc-TALC increased H3K27M expression by mediating the acetylation of H3K9, H3K27 and H3K36 in MGMT promoter regions through the c-Met/Stat3/300 axis. In clinical patients, Inc-TALC is required for TMZ resistance and GBM recurrence. Our results reveal that Inc-TALC could serve as a therapeutic target to overcome TMZ resistance, enhancing the clinical benefits of TMZ chemotherapy.

**TBI-24. USING MOLECULAR GUIDED THERAPY IN PEDIATRIC NEURO ONCOLOGY PATIENTS: THE SUCCESS AND BARRIERS**

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Remarkable advances have been made in pediatric brain tumor treatments, however many of these children suffer significant side effects from standard chemotherapy. Radiation and adjuvant chemotherapy offer great hope to pediatric patients in terms of improved therapeutic precision, safety, and efficacy. However, there are barriers to implementing precision medicine that are best approached from a multi-disciplinary perspective. The goals of the Riley Hospital for Children at Indiana University Health Precision Genomics Neuro Oncology program are to optimize the treatment of children by assessing children’s cancers for actionable molecular targets and finding available, affordable therapeutic options. We review those actionable targets, including lncRNA expression, as well as Precision Genomics Neuro Oncology program at the time of diagnosis or with relapse. Tumor tissue is tested for somatic and germline findings. Riley’s Precision Genomics Neuro Oncology program has received 35 patient referrals. Of these 35 patients, 46 (84%) had molecular analysis completed, and the results of 40 (87%) patients indicated actionable tax.

**TBI-19. INTEGRATED GENOMIC, PROTEOMIC AND PHOSPHOSPROTEOMIC ANALYSIS OF SEVEN TYPES OF PEDIATRIC BRAIN CANCER**

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We performed a comprehensive proteogenomic analysis across seven childhood brain tumors for a deeper understanding of their functional biology. Whole genome sequencing, RNAseq, quantitative proteomic profiling, and phosphoproteomic analyses were performed on 246 preclinical samples representing the histologic diagnoses of: low grade astrocytoma (93), ependymoma (32), high grade astrocytoma (26), medulloblastoma (22), ganglioglioma (18), craniopharyngioma (16) and atypical teratoid rhabdoid tumor (12). Unsupervised clustering analysis based on proteomics data reveals eight clusters with distinct protein profiles and pathway activities. While some clusters coincide with histologic diagnoses, a couple of clusters appear to be a mixture of different diagnoses, including one cluster consisting of “aggressive” tumors characterized by poor survival and high stemness scores. By integrating proteomic data with RNAseq and WGS data, we characterize the impact of mutations (H3K27M, BRAFV600E, BRAF fusion) and CNVs upon the proteome across various diagnoses. Multitomics based kinase-substrate association analysis and co-expression network analysis reveal targetable active kinase networks within these tumors. Proteomic data reveals unique biology associated with H3K27M mutation status in HGG and BRAF aberrations in LGG. Characterization of the tumor microenvironment through deconvolution analyses based on multi-omics data reveals tumor cluster associations with different populations of infiltrating immune cells and the relative activity of the immune system based upon the expression of pro-inflammatory or immunosuppressive markers. This study reports the first large-scale deep comprehensive proteogenomic analysis of pediatric brain tumors using a translational, multi-omic approach. Recent advances in precision pediatric brain tumor biology including functional insight that helps drive translational efforts.
Fueyo, Alan
Boston Children’s Hospital/Dana-Farber Cancer Institute, and Harvard Medical School, Boston, MA, USA, Becher, Krug, Louati, Goodale, Jaime Gallego Perez de O’Sullivan, Piccioni, Hambardzumyan, Patiño-García, Abdelmoula, Li, Storer, and Laspidea.

Abstracts

Molecular targeted therapies are currently being used to treat children with cancer, and the challenges involved.

TBIO-26. NON-CANONICAL OPEN READING FRAMES ENCODE FUNCTIONAL PROTEINS ESSENTIAL FOR CANCER CELL SURVIVAL

John Premont1, Oana Enache2, Victor Luria1, Karsten Krug3, Karl Clauer2, Joshua Dempster4, Amir Karger5, Li Wang6, Karolina Stumbrate2, Vickie Wang7, Genevra Botta8, Nicholas Lyons9, Amy Goodale1, Zehra Kalani, Briana Fritchman, Adam Brown10, Douglas Allen11, Xiaoping Yang12, Jacob Jaffe13, Jennifer Roth2, Federica Piccinii14, Marc Kirschner15, Zhe Ji16, David Root17, and Todd Golub18,19.

The brain is the foremost non-gonadal tissue for expression of non-coding RNAs of unclear function. Yet, whether such transcripts are truly non-coding or rather the source of non-canonical protein translation is unknown. Here, we used functional genomic screens to establish the cellular bioactivity of non-canonical proteins located in putative non-coding regions of transcriptome-wide translated regions of protein-coding genes. We experimentally interrogated 553 open reading frames (ORFs) identified by ribosome profiling for three major phenotypes: 27% (46%) demonstrated protein translation when ectopically expressed in HEK293T cells, 40% (77%) induced a significant increase in cell survival and a significant increase in cell survival and 37% (10%) induced a viability defect when the endogenous ORF was knocked out using CRISPR/Cas9 in 9 human cancer cell lines. CRISPR/Cas9 allowing and start codon mutagenesis increased the number of identified ORFs. Given that the biological impact of these non-canonical ORFs requires their translation as opposed to RNA-mediated effects, we functionally characterized one of these ORFs, G029442−renamed GREP1 (Glycine-Rich Extracellular Protein 1)—as a cancer-implicated gene with high expression in human cancer cell lines, including gliomas. GREP1 knockdown in >200 cancer cell lines reduced cell viability in multiple cancer types, including glioblastoma, in a cell-autonomous manner and produced cell cycle arrest via single-cell RNA sequencing. Analysis of the secretome of GREP1-expressing cells showed increased abundance of the oncogenic cytokine GDF15, and GDF15 supplementation mitigated the growth inhibitory effect of GREP1 knock-out. Taken together, these experiments suggest that the non-canonical ORFome is surprisingly rich in biologically active proteins and potential cancer therapeutic targets deserving of further study.

TBIO-27. RASOPATHIES AND BRAIN TUMORGENESIS: ARE SOS1 MUTATIONS CONCERNED?

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Germ line gain-of-function mutations in several members of the RAS/MAPK pathway, including PTPN11 are associated with signalopathies named Rasopathies and known as Noonan syndrome and closely related conditions. Patients harboring Rasopathies are at increased risk of myeloproliferative diseases and solid tumors, such as neuroblastoma. Mutations of SOS1, the gene encoding a guanine nucleotide exchange factor for Ras, represent the second most frequent genetic defect in Rasopathies. However, SOS1 mutations are rare in human malignancies and patients with germline SOS1 mutations may not be at increased risk of developing cancer. Here, we report a SOS1 variant found to segregate in a Tunisian family. Two aunts developed blindness and then died subsequently to leptic conditions as well as recurrent brain malignancies in the paternal family. Two aunts developed blindness and then died subsequently to leptic conditions as well as recurrent brain malignancies in the paternal family. 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