TBIO-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 glioneuronal tumors. Recent publications were highlighting BRAF mutation and CDKN2A deletion, as independent prognostic factors linked to a worst 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 CDKN2A deletion. Two pediatric HGGs relapsed rapidly. Only one benefited positively from targeted therapy. Specific radiological and spectroscopic signs were linked to the BRAF mutation itself and those different groups (LGGs, HGGs and LGGs with long term evolution of HGG), where specific molecular pathways and metabolomic profiles are associated. Currently, we are going further in the correlations to be able to predict in LGG their potential long-term evolution, where MAPK pathway modulations might be involved.

TBIO-18. ESTABLISHING A PIPELINE FOR INDIVIDUALIZED TREATMENT OPTIONS FOR PEDIATRIC BRAIN CANCER

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INTRODUCTION: Despite being able to characterize pediatric brain tumors such as medulloblastoma and high-grade gliomas using detailed molecular analysis tools, this knowledge hasn't been translated to better treatment methods. In this project, we aim to create a biobank of pediatric brain tumors (PBTs), characterize samples using next generation molecular diagnostics and identify patient specific drug-treatment options using high-throughput drug screening (HTDS). METHODS: To establish tumor spheres from biopsies, we mechanically dissociated the tissue and digested it in trypsin. The cells isolated were cultured in serum free DMEM medium. Immunocytochemistry analysis was done to compare the spheres and original tumor. After the second passage, DNA was extracted and subjected to low-pass whole genome nanopore sequencing. HTDS with a library of FDA/EMA-approved anticancer drugs and investigational compounds was also performed. RESULTS: We've established tumor sphere cultures that grew to passage two and onwards from five juvenile pilocytic astrocytomas, two gangliogliomas and two midline gliomas. The spheres expressed markers of stem cells (Nestin), neurons (β3-tubulin) and glial (GFAP), similar to the original tumor. Copy number profiling and methylation-based classification of the spheres showed the same alterations and classification as the biopsy. HTDS revealed significant differences in drug sensitivity including patient-specific vulnerabilities to anticancer drugs. CONCLUSION: We've created a protocol to generate tumor spheres from PBTs. We are also building a biobank comprising high and low grade PBTs. Our tumor spheres maintain the characteristics of the original tumor and can be used for further downstream analysis including drug screening.

TBIO-19. INTEGRATED GENOMIC, PROTEOMIC AND PHOSPHOPROTEOMIC 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 phosphoproteomics were performed on 219 fresh frozen tumor 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. Multiomics 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 5 distinct tumor clusters associated with different populations of infiltrating immune cells and the relative activity of the immune system based upon the expression of pro-inflammation or immunosuppressive markers. This study reports the first large-scale deep comprehensive proteogenomic analysis crossing traditional histologic boundaries to uncover foundational pediatric brain tumor biology including functional insight that helps drive translational efforts.

TBIO-21. LNC-TALC PROMOTES O^6-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 cells, and find an unreported lncRNA in GBM, lnc-TALC (temozolomide-associated 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 in TMZ-resistant GBM cells. Furthermore, lnc-TALC increased MGMT expression by mediating the acetylation of H3K9, H3K27 and H3K36 in MGMT promoter regions through the c-Met/Stat3/p300 axis. In clinical patients, lnc-TALC is required for TMZ resistance and GBM recurrence. Our results reveal that lnc-TALC in GBM could serve as a therapeutic target to overcome TMZ resistance, enhancing the clinical benefits of TMZ chemotherapy.

TBIO-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-based treatment. Recent advances in precision medicine 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 multidisciplinary 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 therapies that treat those actionable targets. Children are referred to the Riley 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 55 patient referrals. Of these 55 patients, 46 (84%) had molecular analysis completed, and the results of 40 (87%) patients indicated actionable tar-