TBIO-12. THE SPECTRUM OF MITOCHONDRIAL DNA (MTDNA) MUTATIONS IN PEDIATRIC CENTRAL NERVOUS SYSTEM (CNS) TUMORS

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To explore the role of mitochondrial DNA mutations in pediatric CNS tumors, we analyzed 749 tumor-normal paired whole genome sequencing data sets from the Children's Brain Tumor Tissue Consortium (CBTTC). We detected 307 somatic mtDNA mutations in 222 CNS tumors (29.6%). Most frequently observed were missense mutations (38.1%). We also detected 34 loss-of-function mutations. Different pediatric CNS tumor subtypes have distinct mtDNA mutation profiles. For categorical comparisons, we analyzed subtypes with at least 15 samples. The highest number of mtDNA mutations per tumor sample was in meningiomas (0.85), while atypical teratoid rhabdoid tumors (ATRTs) had the lowest number per sample (0.18). High-grade gliomas had a higher number of mtDNA mutations per sample than low-grade gliomas (0.56 vs. 0.31) ($p = 0.0011$), with almost twice as many missense mtDNA mutations per sample (0.22 vs. 0.13) ($p < 0.001$), and higher average heteroplasmy levels (11% vs. 9%). The average heteroplasmy was 10.1%, ranging from 15.6% in medulloblastoma to 6.36% in schwannoma suggesting that these are clonal alterations and not artifacts. Intriguingly, the two chordoma patients in the CBTTC database had an identical heteroplasmic m.10971G>A *MT-ND4* nonsense mutation. Similarly, our patient with recurrent gliofibroma harbored the same somatic *MT-ND4* synonymous variant (m.10700A>G) detected at 53% heteroplasmy in the initial tumor, 79% in the first recurrence, and 97% in the second recurrence. Although the functional consequences of these alterations are not yet understood, our findings suggest that sequencing the mtDNA genome may be used to characterize CNS tumors at diagnosis and monitor disease progression.

TBIO-13. USE OF NEXT GENERATION SEQUENCING TO IDENTIFY MOVE DRIVERS OF CRYPTIC, CLINICALLY AGGRESSIVE BRAIN TUMORS

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INTRODUCTION: Next generation sequencing (NGS) is an emerging technology which allows for in-depth analysis of pediatric brain tumors. NGS has particular use in the context of ambiguous or aggressive neoplasms, where it can be leveraged to discover novel drivers, inform pathologic classification, and direct targeted therapies. OBJECTIVE: The objective of this case series was to utilize NGS technology to illuminate the biology of aggressive brain tumors with ambiguous pathologic features and clinically aggressive behavior. METHODS: FFPE tumor tissue and matched germline DNA were subjected to whole exome sequencing (WES). Data were analyzed according to the GATK pipeline. RESULTS: The first case is a 6-year-old male who presented with innumerable foci of leptomeningeal nodules throughout the neuroaxis. Original pathology was CNS embryonal tumor. WES identified loss of chromosome 1p and 16q with gain of 1q and amplification of *MYC* and *OTX2* loci (cytogenetic aberrations characteristic of group 3 medulloblastoma) and a deleterious mutation in *BCL7B*, a known tumor suppressor gene. The second case is a 2-year-old female who presented with a parietal lobe mass diagnosed as high grade neuroepithelial tumor with *C11orf95* translocation, but no *RELA* fusion. WES revealed loss of small region of chromosome 2p and mutations in *IDH3G*, *TRAF2,* and *JMJD1C*, suggesting novel targets for further study. CONCLUSIONS: In both cases, NGS studies were able to shed light on the underlying tumor biology and/or refine the pathologic diagnosis. These data underscore the utility of applying NGS technology to study the biology of pediatric brain tumors.

TBIO-14. CHARACTERISATION OF THE ARGININE PATHWAY ENZYMES IN PAEDIATRIC BRAIN TUMOURS TO DETERMINE SUSCEPTIBILITY TO THERAPEUTIC ARGININE DEPLETION

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INTRODUCTION: Extracellular arginine dependency (auxotrophy) is increasingly being recognised in several tumours. This is due to the inability of cancer cells to recycle or synthesise intracellular arginine through the urea cycle pathway compared to normal cells. Whilst adult glioblastoma is known to exhibit this, the expression of the arginine pathway enzymes has not been delineated in paediatric brain tumours. METHODS: We used immunohistochemical methods to stain for arginine pathway enzymes in Paediatric High grade glioma (pHGG), low grade glioma (pLGG) and medulloblastoma (MB) tumour tissue microarrays (TMAs). The antibodies detected protein expression of the metaboliser Arginase (Arg2), recycling enzymes ornithine transcarbamoylase (OTC), Arginosuccinate synthetase (ASS1) and arginosuccinate lyase (ASL) as well as the transporter SLC7A1. RESULTS: Deficiency of OTC, ASS1 and ASL were seen in 92%, 98% and 93% of pHGG samples (n=156) respectively, with deficiency defined as low (<20%) or negative antibody expression. Identical results were seen in pLGG (n=98) - 83%, 97% and 95% were deficient in OTC, ASS1 and ASL. Both pHGG and pLGG highly expressed SLC7A1 and Arg2, demonstrating that they could transport and utilise arginine. In MB (n=82), this auxotrophic signature was again seen in 90% of TMAs with absent or low expression of OTC, ASS1 and ASL and high Arg2 and SLC7A1 expression. CONCLUSIONS: These results show that pHGG, pLGG and MB are arginine auxotrophs. Pegylated arginase (BCT-100) is currently in Phase I/II trials in relapsed pHGG. Our results suggest that therapeutic arginine depletion may also be useful in MB and pLGG.

TBIO-15. MODELING DEVELOPMENTAL GENE EXPRESSION DYNAMICS AT CELLULAR RESOLUTION TO INTERPRET PEDIATRIC BRAIN TUMOR TRANSCRIPTIONAL PROGRAMS

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A central challenge in understanding the biology of pediatric brain tumors is defining the cellular and molecular context where oncogenesis occurs. We hypothesize that spatiotemporally restricted cell types are uniquely susceptible to specific genetic alterations, which alter normal neurodevelopmental programs and ultimately lead to oncogenesis. The resulting tumors retain some transcriptomic features of their lineage of origin. To delineate these origins, we assembled a densely sampled developmental time course of the mouse forebrain and pons, doubling our recently published single-cell atlas. This dataset comprises >100,000 cells at 9 timepoints from E10-P6. However, while single cell transcriptomics reveal rich gene dynamics during cell differentiation, interpretation of individual genes can be challenging due to data sparsity. Leveraging this time-series, we present strategies to model and visualize the expression of a given gene across differentiation of distinct lineages. We demonstrate an interactive web app to interrogate the expression of genes or gene sets during brain development, extract temporally correlated genes, and search active transcription factor regulatory modules. Finally, we profile the expression of core transcriptional programs of several pediatric brain tumor entities during development. Our analyses reveal genes with restricted expression patterns that elucidate tumor etiology. More broadly, these resources harness single cell data to enable exploration of neurodevelopmental gene programs with great relevance to pediatric brain tumor oncogenesis.

TBIO-16. NOTCH1 PATHWAY AS TARGET FOR DRUG INTERVENTION FOR HISTONE 3 G34R MUTATED PHGG

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There have been no significant improvements in the treatments for childhood High-Grade Glioma (pHGG) and Diffuse Intrinsic Pontine Glioblastoma (DIPG), which continue to have a very poor prognosis. These cancers harbor mutations affecting histone 3 (H3) proteins; 80% of DIPGs with histone H3 K27M somatic mutations whilst 30% of pHGGs exhibit H3.3 G34R or G34V mutations. We have generated and validated a histone 3.3 G34R mutant-specific antibody and investigated the downstream effects of H3.3 G34R mutations in pHGG. In order to identify the genes that may be deregulated by G34R mutant histone expression, we have performed chromatin immunoprecipitation (ChIP) assays with our H3.3 G34R and wild type H3 antibodies, using pHGG H3 G34R mutant and wild-type cell lines. Initial analyses of ChIP data have implicated deregulation of cell-signaling pathways including Notch1, Hedgehog, PPAR-1, PLC-beta and Androgen, in H3 G34R mutated pHGG. We are currently determining the effects of altered expression of Notch1 pathway components on tumorigenesis of H3 G34R mutated pHGG, through gene and protein expression and inhibition assays. Specifically we find that the Notch1 pathway component HES1 shows increased expression in G34R mutant cells compared to controls, directing our evaluation of the utility of gamma-secretase inhibitors as potential therapeutics. These analyses may underpin development of novel treatment strategies for H3 mutated pHGG.