tumors 3.4%) compared to nontumor tissues (5.3%). We subset to the CpGs with the 5% highest 5hmC content for downstream analyses (37,173 CpGs). These sites were enriched among regulatory elements, including TFBS (Odds Ratio 1.14 p-value 3.57E-20) and super-enhancers (OR 1.93, p-value 1.14E-126). Linear mixed-effects models adjusted for age, sex, and cell type proportions tested the CpG-specific differences in 5hmC between tumor and nontumor samples, as well as between tumor subtypes. 5hmC levels were depleted across tumors compared with nontumor brain tissues, including at CpG islands. Model-based clustering (RPMM) results indicated that patients with low 5hmC patterns have poorer overall survival and increased risk of recurrence. Our results indicate that 5hmC localizes to sites in the DNA critical to gene regulation and is associated with patient outcomes. This study offers an opportunity to potentially contribute to classification markers for childhood brain tumors.

TBIO-06. BDNF-TRKB SIGNALING REGULATES NEURON-GLIOMA SYNAPTOGENESIS AND PROMOTES TUMOR PROGRESSION

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Pediatric high-grade gliomas (pHGG) are a devastating group of diseases that urgently require novel therapeutic options. We have previously demonstrated that pHGGs hijack mechanisms of brain development and plasticity to their advantage. Here, we investigated the role of microenvironmental BDNF on pediatric gliomas, independent of the NTRK fusion events commonly identified in infant HGG. Genetic deletion or pharmacological blockade of *NTRK2* (TrkB), in patient-derived pediatric glioma increases survival in multiple DIPG and pGBM patient-derived orthotopic xenograft models. Unlike the paracrine BDNF-TrkB signaling observed between subpopulations of adult HGG malignant cells, pediatric glioma express TrkB, but not BDNF ligand. BDNF is secreted by normal brain cells in response to neuronal activity and conditioned medium experiments from cortical slices of mice indicates the brain microenvironment as the chief source of BDNF ligand. Addition of recombinant BDNF protein increases pediatric glioma cell proliferation and activates the canonical downstream MAPK signaling pathway, an effect that is blocked by genetic or pharmacological TrkB inhibition in pHGG. However, the glioma growth-promoting effects of BDNF in vivo cannot be explained by stimulation of MAPK signaling alone. We therefore examined the effects of BDNF signaling on neuron-to-glioma synapse formation, a newly recognized microenvironmental interaction important for pediatric glioma progression. We find that BDNF-TrkB signaling promotes neuron-to-glioma synaptogenesis in neuron-glioma co-culture. We are presently exploring the role for BDNF-TrkB signaling in glioma synaptic plasticity and function. Funding: Abbie's Army Foundation

TBIO-07. SINGLE-CELL TRANSCRIPTOMIC PROFILE REVEALS MACROPHAGE HETEROGENEITY IN SONIC-HEDGEHOG MEDULLOBLASTOMA AND THEIR DISTINCT RESPONSES TO DIFFERENT TREATMENT MODALITIES

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Tumor-associated macrophages (TAMs) are an important component of the tumor microenvironment. Pro-inflammatory macrophages can suppress while anti-inflammatory macrophages can promote tumor growth. Despite their abundance in many tumors, the origins and diversity of TAMs are not well understood, especially in pediatric brain tumors. Using single-cell RNA sequencing in a genetically engineered mouse model (Ptch+/-:p53-/-) of SHH-MB, we identified the dual microglia and monocytic origin of macrophage and their transcriptomic heterogeneity. We demonstrate differential recruitment and function of macrophages under distinct modalities of tumor therapy of molecular targeted hedgehog inhibition versus radiation. We additionally identify a monocytic macrophage population recruited post-radiation that is immune suppressive, suggesting a mechanism for radiation treatment failure. These insights uncover potential strategies for immunomodulation as adjunctive therapy for radiation.

TBIO-08. BASE-RESOLUTION METHYLOMES OF GLIOMAS BEARING HISTONE H3.3 MUTATIONS REVEAL A G34 MUTANT-SPECIFIC SIGNATURE SHARED WITH BONE TUMORS

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BACKGROUND: Two recurrent mutations, K27M and G34R/V, in H3F3A, encoding non-canonical histone H3.3, are reported in pediatric and young adult gliomas, whereas G34W mutation was prevalent in bone tumors. In contrast to K27 mutation, it remains elusive how G34 mutations affect the epigenome. Here we performed whole-genome bisulfite sequencing of four G34R-mutated gliomas and the G34V-mutated glioma cell line KNS-42. Similarly, we analyzed seven and three gliomas harboring K27M and no mutations in H3F3A, respectively. These data were compared with those on bone tumors. RESULTS: G34R-mutated gliomas exhibited lower global methylation levels, similar CpG island (CGI) methylation levels, and compromised hypermethylation of telomere-proximal CGIs compared with those bearing K27M and no mutations. Hypermethylated regions specific to G34R-mutated gliomas were enriched for CGIs, including those of OLIG1, OLIG2, and canonical histone genes in the HIST1 cluster. These CGIs were hypermethylated in osteosarcomas with, but not without, the G34W mutation. In KNS-42 cells, CGIs with G34V-mutated histone H3.3 exhibited higher methylation levels than those with wild-type histone H3.3. This effect was also observed in the G34R-mutated glioma samples. CONCLUSIONS: Gliomas bearing G34R/V mutations display characteristic methylomic alterations, some of which are shared by osteosarcomas with the G34W mutation. Deposition of G34 variants may lead to elevated methylation of otherwise hypomethylated, histone H3.3-bearing CGIs.

TBIO-09. *IN SILICO* ANALYSIS IDENTIFIES A PUTATIVE CELL-OF-ORIGIN FOR *BRAF* FUSION-POSITIVE CEREBELLAR PILOCYTIC ASTROCYTOMA

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Childhood cancers are increasingly recognized as disorders of cellular development. This study sought to identify the cellular and developmental origins of cerebellar pilocytic astrocytoma, the most common brain tumor of childhood. By leveraging publicly available gene expression data from such tumors and controlling for driver mutations, a set of eight known neuro-developmental genes were identified as being upregulated in cerebellar pilocytic astrocytoma. Mapping those genes onto mouse neurodevelopmental atlases identified significant overlap in their expression within the ventricular zone of the cerebellar anlage. Further analysis with a single cell RNA-sequencing atlas of the developing mouse cerebellum defined this overlap as occurring in ventricular zone progenitor cells at the division point between GABA-ergic neuronal and glial lineages, a developmental trajectory which closely mirrors that previously described to occur within pilocytic astrocytoma cells. Furthermore, ventricular zone progenitor cells and their progeny exhibited evidence of MAPK pathway activation, the paradigmatic oncogenic cascade known to be active in cerebellar pilocytic astrocytoma. Gene expression from developing human brain atlases recapitulated the same anatomic localizations and developmental trajectories as those found in mice. Taken together, these data suggest this population of ventricular zone progenitor cells as the cell-of-origin for *BRAF* fusion-positive cerebellar pilocytic astrocytoma.

TBIO-11. DEEP LEARNING-BASED SINGLE-CELL RNA SEQUENCING DIFFERENTIATION IDENTIFIES SIMPLE AND COMPLEX TRANSCRIPTIONAL NETWORKS FOR SUBPOPULATION CLASSIFICATION

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BACKGROUND: Genomic assays capable of cellular resolution (i.e. scRNA-seq) are becoming ubiquitous in biomedical research. Machine learning, and the subtype known as Deep Learning, have broad application within scRNA-seq analytics. However, methods to facilitate the classification of cell populations are lacking. We present the novel computational framework HD Spot, which generates interpretable and robust Deep Learning classifiers that enable unbiased interrogation of linear and non-linear genomic signatures. METHODS: HD Spot is written in python and relies on Google's TensorFlow2 deep learning framework. Four datasets of immune cells were obtained from the publicly available Seurat repository, generated using the 10X chromium platform. Data preprocessing used standard Seurat methodology. HD Spot generated optimized classifiers via a custom platform. Network interpretability was achieved using Shapley values. Ontology analysis was performed using Metascape. RESULTS: HD Spot identified meaningful ontologic signatures across all tested datasets. In the binary case of control versus IFN-B stimulated CD4+ T cells, gene ontologies reflected T_{h0} and T_{h2} T cell populations, congruent with T cell activation. In the 9-class case of PBMCs, HD Spot identified meaningful gene networks characteristic of the ground-truth populations using raw feature counts alone. When feature counts are processed into expression values, HD Spot demonstrates increased specificity of top genes and respective ontologies between subpopulations. CONCLUSION: This work introduces a broadly applicable computational tool for the advanced bioinformatician to decipher complex cellular heterogeneity (e.g., tumors) in an unbiased way. Additionally, HD Spot lowers the barrier for novice bioinformaticists to derive actionable insights from their data.