TUMOR BIOLOGY (NOT FITTING A SPECIFIC DISEASE CATEGORY)

TBIO-01. SEX DIFFERENCES IN REDOX STATE UNDERLIE GLUTAMINE DEPENDENCY IN MALE Glioblastoma
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Glioblastoma (GBM) is an aggressive brain tumor in children and adults. It occurs more commonly in males, but female patients survive significantly longer. Understanding the molecular mechanisms that underlie these sex differences could support novel treatment strategies. In this regard, we found that male and female GBM patient samples differ in their metabolite abundance and that males exhibit a significantly higher abundance of amino acid metabolites. We confirmed these findings in a murine model of GBM, which has previously yielded important insights into sexual dimorphism in GBM. Furthermore, we found that male GBM cell cultures are significantly more sensitive to amino acid deprivation, which was almost entirely driven by amino acids involved in the synthesis of the antioxidant glutathione. Glutaminase 1 (GLS1) mediates the conversion from glutamine to glutamate, a crucial component of glutathione. We found that male GBM cells exhibited higher levels of GLS1, suggesting they are more dependent on glutamate. In contrast, we found that male GBM cells are more sensitive to pharmacological GLS1 inhibition with the clinical inhibitor CB-839. This correlated with significantly increased reactive oxygen species (ROS) in males compared to females. We further confirmed sex differences in redox state through pharmacological depletion of glutathione that resulted in a significant increase in ROS and cell death in male GBM. Together, these data indicate that male GBM cells are more dependent on glutamine to regulate ROS levels. This reveals novel sex-specific metabolic targets for GBM and underlines the importance of considering sex in metabolic targeting approaches.

TBIO-02. IMMUNE PROFILING OF RARE EMBRYONAL BRAIN TUMORS REVEALS EVIDENCE OF DYSREGULATED INTERFERON PATHWAYS AS A POTENTIAL DETERMINANT OF IMMUNOLOGICAL HETEROGENEITY
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Embryonal brain tumors (EBTs) remain the most common malignant pediatric brain tumors. Despite recent advances and improved understanding of the molecular biology of EBTs, clinical outcomes remain poor for rare EBTs. Previous large-scale genomic studies of rare EBTs have shed light on distinct genomic, transcriptomic and epigenomic profiles. Interestingly, recent studies have revealed prominent tumor heterogeneity, providing opportunity to develop novel treatment strategies to improve patient outcomes. To examine the tumor microenvironment and identify tumor-specific biological dependencies, we performed deconvolution analysis of bulk gene expression (171 RNA-seq) and 586 methylated arrays, which revealed significant intra- and inter-tumoral heterogeneity and implicated interferon (IFN)-mediated signalling as a determinant of a distinct immunological profile in rare EBTs. To further elucidate the importance of IFN signalling, we performed chIP-sequencing on 20 primary samples, which provided evidence of IFN-immunological responses that vary from immunosuppressive to immunologically exhaustive that occur in a host-dependent manner. To further validate our findings, we utilized a genetically engineered murine model of Arpical Teratoid Rhabdoid Tumor and primary patient-derived mouse and in-silico profile data in vivo. Through amalgamation of our in-silico data with our in vivo data, we have identified evidence that dysregulated IFN responses represent a core element of the immunological heterogeneity present within subsets of rare EBTs. An improved understanding of the immune milieu in rare EBTs will provide avenues to develop specific onco-immune targets to address this clinical need.

TBIO-03. THE GIFT FROM A CHILD PROGRAM IS EMPOWERING POST-MORTEM TISSUE DONATION ACROSS THE UNITED STATES
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The Gift from a Child (GFAC) program was inspired by the dream of one child to donate his brain for research, recognizing the need to study tumor tissue collected at diagnosis, recurrence, and at the time of death. Founded by the Swifty Foundation in 2016, GFAC currently is comprised of five “Centers of Excellence” at institutions with expertise in pediatric neuro-oncology. Partnering with the Children’s Brain Tumor Network, the program’s mandate is twofold: make it possible for families to donate no matter where they live in the United States and make tissue available to scientists globally to empower discovery. In order to overcome barriers that have stifled postmortem collection in the past, GFAC has invested in Tissue Navigators - individuals at each center who coordinate all aspects of donation and communicate with families, medical providers, and laboratory scientists. In 2019 alone, GFAC coordinated 53 donations from multiple diagnoses. A key metric of the program is also capturing the global sharing and usage of each tissue sample, ensuring that tissue isn’t simply “banked” but is actively being used to help unravel tumor biology. To do this, GFAC has engaged with genomic and molecular experts, bolstered preclinical model development including cell lines and PDX models, and for novel drug screening. Together with Children’s Brain Tumor Network, the Gift from a Child program is helping to ensure the most precious gift that a family can make is used to accelerate the path to cures.

TBIO-05. GENOME-SCALE NUCLEOTIDE-SPECIFIC CHARACTERIZATION OF 5-HYDROXYMETHYL-CYTOSINE IN PEDIATRIC CENTRAL NERVOUS SYSTEM TUMORS
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Though aberrant cytosine modifications are prevalent in cancer, nucleotide-specific 5-hydroxymethylcytosine (5hmC) modifications remain understudied, including in pediatric CNS tumors. Brain 5-hydroxymethylation is linked with development and differentiation. We measured genome-scale 5hmC in pediatric brain tumors with diagnoses of diffuse intrinsic pontine gliomas and embryonal tumors under age 18 (n=36), and in non-tumor pediatric brain tissues (n=3). DNA was processed with tandem oxidative (OxBS) and bisulfite (BS) treatments followed by hybridization to the Illumina Methylation EPIC Array that interrogates over 600,000 CpG sites. We used the OxBS package to determined levels of 5hmC and 5mC. Mean 5mC levels were lower in tumors (gliomas 4.1%, epidermodymas 3.9%), and embryonal

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Pediatric high-grade gliomas (pHGGs) 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 glioma models increases survival in mutant p53 and p16/CDKN2A and p53/CDKN2A-p53-/- tumors. 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 synaptogenesis, formation of a synaptology which closely mirrors the neuro-developmental genes were identified as being upregulated in cerebellar pilocytic astrocytoma. Gene expression from developing human brain atlases recapitulated the same anatomic localizations and development trajectories as those found in mice. Taken together, these data suggest this population of ventricular zone progenitor cells as the cell-of-origin for 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. METHODO: HD Spot is written in python and relies on Google’s Tensorflow 2 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 and 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-β treated CD14+ T cells, gene ontologies reflected Treg and Th17 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 recruits novel 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 bioinformaticians to derive actionable insights from their data.