Communication between cancer cells and immune cells is a key determinant of the glioblastoma ecosystem and its response to therapies, but remains incompletely understood. Here we leveraged single-cell RNA-sequencing (scRNA-seq) of human samples and mouse models, deconvolution analysis of bulk specimen from The Cancer Genome Atlas (TCGA) and functional assays, to dissect cellular cross-talks in glioblastoma. We demonstrate that glioblastoma renders a transition of glioblastoma cells into mesenchymal-like (MES-like) states. This effect is mediated, both in vitro and in vivo, by macrophage-derived Oncostatin M (OSM) and its cognate receptor OSMR on glioblastoma cells. We show that MES-like glioblastoma states are also associated with increased expression of a mesenchymal program in macrophages and with increased cytotoxicity of T cells, highlighting extensive alterations of the immune microenvironment with potential therapeutic implications.

OTME-8. INHIBITORY CD161 RECEPTOR IDENTIFIED IN GLIOMA-INFILTRATING T CELLS BY SINGLE-CELL ANALYSIS
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T-cells are critical effectors of cancer immunotherapies, but little is known about T-cell gene expression programs in diffuse gliomas. We leveraged single-cell RNA-seq to chart the gene expression and clonal landscape of tumor-infiltrating T-cells across 31 patients with isocitrate dehydrogenase (IDH) wild-type glioblastoma and IDH mutant gliomas. Our analysis revealed subsets of T-cells that expressed several NK-cell receptors, in particular the inhibitory CD161 receptor (KLRB1 gene). KLRB1 was overexpressed by clonally expanded CD8 T-cells, and larger populations of T-cells expressed CD161 than PD-1. The CLEC2D ligand of CD161 was expressed by malignant cells and myeloid cells, and inactivation of KLRB1 enhanced anti-tumor T-cell function. KLRB1 was also expressed by substantial NK-cell populations in multiple glioma cancers. CD161 and other NK-cell receptors expressed by T-cells represent opportunities for immunotherapy of diffuse gliomas and other human cancers.

OTME-9. COMPREHENSIVE METABOLIC PROFILING OF HIGH MYC MEDULLOBLASTOMA REVEALS KEY DIFFERENCES BETWEEN IN VITRO AND IN VIVO GLUCOSE AND GLUTAMINE USAGE
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Reprogramming of cellular metabolism is a hallmark of cancer. The metabolic alterations in cancer cells is not only defined by series of genetic mutations, but also reflecting the crosstalk between cancer cells and other factors in the microenvironment. Altering metabolism allows cancer cells to overcome unfavorable conditions, to proliferate and invade. Medulloblastoma is the most common malignant brain tumor of children. Genomic amplification of MYC is a hallmark of a subset of poor-prognosis medulloblastoma. However, the metabolism of high MYC amplified medulloblastoma subgroup remains unexplored. We performed comprehensive metabolic studies of human MYC-amplified medulloblastoma by comparing the metabolic profiles of tumor cells in different environments - in vitro, in flank xenografts and in orthotopic xenografts. Principal component analysis showed that the metabolic profiles of brain and flank high-MYC medulloblastoma tumors clustered closely together and separated away from normal brain and the high-MYC medulloblastoma cells in culture. Compared to normal brain, MYC-amplified medulloblastoma orthotopic xenograft tumors showed upregulation of nucleoside, hexosamine bio-synthetic pathway (HBP), TCA cycle, and amino acid and glutathione pathways. There was significantly higher glucose uptake and usage in orthotopic xenograft tumor compared to flank xenograft and cells in culture. The data demonstrated that glucose was the main carbon source for the glutamate, glutathione and glutathione synthesis through the TCA cycle. The glutaminase pathway was the main pathway utilizing glutamine in MYC-amplified medulloblastoma in vivo. Glutathione was found to be the most abundant upregulated metabolite. Glutamine derived glutathione was mainly synthesized through glutamine transaminase K (GTK) enzyme in vivo. In conclusion, we demonstrated that high MYC medulloblastoma alters the metabolic environments by altering its metabolic pathways despite carrying the same genetic mutations. Glutamine antagonists may have therapeutic applications in human patients.

OTME-10. INTEGRATED ANALYSIS OF HUMAN GLIOMAS AT THE SINGLE CELL LEVEL IDENTIFIES S100A4 AS A NOVEL IMMUNOTHERAPY TARGET
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Understanding the immune composition of a given tumor is critical to assess its potential responsiveness to cancer immunotherapy. This is especially true for tumors that are intrinsically resistant to immunotherapies, such as GBM. Unfortunately, studies on the functional heterogeneity and associated molecular targets of immune-suppressive cellular phenotypes have been lacking. Here we report an integrated multi-dimensional analysis of the mutational profiles and single-cell transcriptomics of 60,024 glioma andstromal cells from 16 human samples. We identified molecular signatures of several distinct macrophage subtypes, each with prognostic clinical value. The three inflammatory subtypes showed hallmarks of TNFβ+NFκB pathway enrichment and are associated with good outcomes; in contrast, four immunosuppressive subtypes with metabolic pathway hallmarks (oxidative phosphorylation, PI3K/AKT/mTOR, fatty acid metabolism) are associated with poor survival. In addition, we resolved an ongoing controversy in the field regarding the roles of brain resident microglia, microglia, vs. bone marrow derived macrophages (BMDM) in gliomas. Our data show compelling evidence that microglia are pro-inflammatory and are associated with good survival with BMDMs are mostly immune-suppressive and associated with poor survival. In addition, deciphering immune-suppressive macrophage and Treg molecular signatures enabled us to identify previously unknown immunotherapy targets. In a proof of principle study, we showed that S100A4, a calcium binding protein previously shown to be upregulated in both innate and adaptive immune suppressor cells, and implantation of gliomas in S100a4−/− host mice significantly extended survival and resulted in pro-inflammatory immune landscape, compared to same glioma cells implanted in syngeneic hosts. This functional validation study shows that S100A4 is a highly promising therapeutic target for GBM immunotherapy.

OTME-11. CHARACTERIZING THE IMMUNOLOGIC CONTEXT OF PEDIATRIC BRAIN TUMORS
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Therapy for pediatric central nervous system (CNS) malignancies can be toxic, and outcomes are suboptimal. Immunotherapy holds promise as a therapeutic avenue, but the poorly understood microenvironment limits its application. The Children’s Brain Tumor Network (CBTN) released the Pediatric Brain Tumor Atlas, containing expression profiles of nearly 700 primary CNS tumors. To study the immune microenvironment, a classification from The Cancer Genome Atlas project is applied. High-grade lesions are predominately lymphocyte deplete (C4, 81%) or immunologically quiet (C5, 11%). Low-grade lesions are more mixed with 46% C4, 21% C5, and a higher proportion of inflammatory subtypes (C3, 31%). For survival, adjusting for tumor grade and extent of resection, the hazard ratio is 2.2 (0.78 – 6.3), p = 0.13) and 2.4 (0.6 – 10.0, p = 0.24) for C4 and C5, respectively. With no events among low-grade tumors, progression-free survival will be another endpoint that is being followed. Additionally, there are gene signatures among C4 samples reveals decreased abundance of T cells (OR 0.26, 0.1 – 0.5) yet increasing T-cell abundance is associated with decreased survival time in high-grade samples (HR 3.7, 1.4 – 10.1). Additionally, there are increased macrophage and decreased microglia signatures among high-grade samples and the C4 and C5 subtypes. It is hypothesized that expression of inhibitory immunomodulators contributes to a pro-tumorigenic microenvironment and represent potential therapeutic targets. In lieu of normal tissue in the data set, differential gene expression experiments between disease states reveals upregulated immunomodulators. Conventional immunomodulators, e.g. PDL1 and CTLA4, are expressed in low-grade samples with C3 subtype, which is abundant in craniopharyngioma. Alternative inhibitory immunomodulators, e.g. IDO, TIM1, EZH2, CD274, are significantly expressed in high-grade samples including diffuse midline glioma. Overall, our analysis contributes to the understanding of the immune microenvironment and identifies potential mechanisms of immune escape among pediatric CNS tumors.

OTME-12. ROLE OF THE TRANSFURLATION PATHWAY IN Glioblastoma Invasion
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Glioblastoma (GBM) is a primary malignant brain tumor with a median survival time under two years. The poor prognosis GBM care is largely due to...