histologically benign, but can cause significant morbidity. Previous studies utilized whole genome and exome sequencing to identify a few somatic variants, but no recurrent mutations were observed. Further studies are warranted to identify driver mutations occurring at low frequencies. We used single-cell RNA sequencing (10X Genomics) to investigate cellular heterogeneity in 12 non-functioning pituitary adenomas. Our analysis identified discrete clusters of cells associated with specific functional pathways. One of these clusters corresponded to cells expressing genes related to metabolic pathways, primarily lipid metabolism. Another cluster consisted amongst the three patients comprised cells involved in antigen presentation and processing. In addition, the copy number variation analysis highlighted distinct chromosomal alterations within our samples. Interestingly, we were able to identify cellular variation within each tumor based on chromosomal aberrations. For example, in our first patient we observed a gain of chromosome 19 and loss of chromosome 2. Our analysis showed three different clonal populations within this tumor. All three populations harbored the loss of chromosome 2, one population exhibited gain of chromosome 19, while a third population exhibited loss of chromosome 19. These early results indicate the loss of chromosome 2 as an early event in tumorigenesis and gain/loss of chromosome 19 as late events. We are currently in a process of identifying somatic variations within these tumors by variant calling. Currently, we are expanding our analysis to 20 non-functional PA. Mapping the single-cell gene expression profiles with mutational phylogeny will reveal the differences in clonal evolution within the tumor subtypes. This study will help us define the molecular fingerprint of pituitary adenomas and provide insights which could be utilized in the clinic for better management of these tumors.

**OTHE-12. ASSESSING ADAPTIVE RESPONSES TO LOSS OF EXTRACHROMOSOMAL DNA AMPLIFICATION**

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**BACKGROUND:** Oncogene activation through somatic gene amplification happens frequently in GBM, with over 70% of these tumors presenting amplification of at least one putative driver gene, oftentimes in small extrachromosomal circular DNA segments composed of chromatin (ecDNA). A molecularly diverse and representative panel of GBM patient-derived cancer stem-like cells (CSC) and orthotopic mouse xenografts (PDX), which retain the original genomic abnormalities and ecDNA amplifications, was employed to assess adaptive response to the absence of ecDNA amplification. METHODOLOGY: We have isolated ecDNA negative cell populations from two patient-derived models. HF3035 harbors a MET amplification and HF2135 harbors a PDGFRα constitutively active genomic rearrangement and extrachromosomal amplification. We conducted paired, whole RNA-sequencing on 20 HF3253 populations (ecDNA+/−: 6 clones from 3 biological replicate PDXs and 4 clones from 4 in vitro technical replicates) and 12 HF3035 population (ecDNA+/−: 6 clones from 3 biological replicate PDXs). RESULTS: Nonparametric differentially expressed gene (DEG) analysis using NOISeqBio (R/ Bioconductor), identified 564 differentially expressed genes (482 upregulated in ecDNA+)/− employing a stringent false discovery rate of 0.05. Genes significantly associated with PDGF stimulation, central cancer metabolism, and H3K27me3 were downregulated in ecDNA+/−, while genes significantly associated with astrocytic processes, neuronal differentiation, and EGFR signaling were upregulated in ecDNA+/− (EnrichR). We employed an additive linear model with PDX serving as a blocking factor to compare ecDNA+ and ecDNA− populations in both models (RedgeR). 2071 genes were upregulated in ecDNA+ PDX specimens and 2365 genes were downregulated. Specifically, ERAP targets were highly enriched in ecDNA+ populations, in addition to mRNA pre-processing. ecDNA loss primarily targeted glycogen metabolism, NTRK signaling, and inositol phosphate catabolism. CONCLUSIONS: We have identified PDX-specific and non-specific features to an adaptive response to the loss of ecDNA amplification. Notably, a signature adaptation is an upregulation of seemingly redundant receptor tyrosine kinases.

**FINAL CATEGORY: OMICS OF TUMOR MICROENVIRONMENT**

**OTME-1. TAMEP ARE BRAIN TUMOR PARENCYHIMAL CELLS CONTROLLING NEOPLASTIC ANGIOPATHY AND PROGRESSION**

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Aggressive brain tumors like glioblastoma depend on support by their local environment and subsets of tumor parenchymal cells may promote specific phases of disease progression. We investigated the glioblastoma microenvironment with transgenic lineage-tracing models, intravital imaging, single-cell transcriptomics, immunofluorescence analysis, and as a histochemical marker characterized by an accumulation of extracellular tumor-associated cells with a myeloid-like expression profile (TAMEP) that transiently appeared during glioblastoma growth. TAMEP of mice and humans were identified with specific markers. Notably, TAMEP did not derive from monocytic cells but were generated from CNS-resident, SOX2-positive progenitors. Abrogation of this progenitor cell population, by conditional Sox2-knockout, drastically reduced glioblastoma vascularization and size. Hence, TAMEP emerge as a tumor parenchymal component with a strong impact on glioblastoma progression.

**OTME-2. REGULATION OF CHROMATIN ACCESSIBILITY IN THE HYPOXIC TUMOR MICROENVIRONMENT OF Glioblastoma**

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**Background:** Tumor microenvironment (TME) of GBM contains brain-residing microglia and bone-marrow derived macrophages (collectively known as glioma-associated microglia/macrophages, GAMS) that constitute up to 30% of the tumor mass and promote tumor invasion. Hypoxia (a shortage of oxygen) is a key factor in tumor progression of GBM as it can globally and rapidly alter the gene expression, induce cancer cell invasiveness, stemness and lead to therapy resistance. Hypoxia can enhance the pro-tumorigenic function of GAMS, e.g. by inducing expression of cytokines and cell surface receptors both in GAMS and glioma cells, but little is known about chromatin alterations of GBM under hypoxia. Since regulation of expression of such molecules could depend on the epigenetic alterations, we hypothesize that hypoxia may potentially alter the chromatin accessibility and functions of GAMS and glioma cells. We determine the genome-wide changes in chromatin accessibility in GAMS and glioma cells in response to hypoxic stress using single-cell Pi-ATAC-seq (Protein-indexed Assay of Transposase Accessible Chromatin with sequencing), which allows simultaneous genomewide assessment of chromatin accessibility and expression of intracellular protein markers in single cells, allowing faithful selection of hypoxic and non-hypoxic cells. Secondly, we are employing an oxygen-dependent co-culture model in vitro to study the mechanisms of chromatin alterations in GAMS and glioma cells under controlled hypoxic conditions and test how these changes depend on the glioma - GAMS cross-communication. In summary, we characterize the interactions between innate immune cells and glioma cells by looking at their chromatin alterations under hypoxic conditions.

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**OTME-3. DISSECTION OF THE ROLE OF STROMAL MICROENVIRONMENT AND TUMOR-TME CROSS-TALK IN PEDIATRIC BRAIN CANCER**

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Brain tumors are the deadliest malignancies that occur during childhood and strong efforts are required to develop innovative therapeutic strategies. The intrinsic capacity of malignant cells to organize, shape and exploit the surrounding environment where they develop (tumor microenvironment, TME), has not been fully elucidated for pediatric brain cancers yet. Here, we exploited a multi-omics approach to define the TME cell populations and their contributions in the most common pediatric brain tumor entities, such as medulloblastomas andependymomas. Analysis of single-cell RNA sequencing data of human tumors resulted in the identification of heterogeneous populations of non-malignant cells present in the TME. In particular, the re-clustering and marker-based cell type assignment strategies allowed to define a broad range of immune and stromal subclones showing distinctive expression signatures reflecting variegated functional roles. By cross-matching the single cell data with normal brain expression atlases, we could further refine the annotation of the newly identified stromal functional subpopulations and define the “tumor-associated” marker signatures of genes exclusively enriched in stromal cells within the TME, linked to immune activation, cell
Epilepsy in the context of brain tumors provides a great burden in these patients, yet mechanisms underlying this process are poorly understood. It has been demonstrated that isocitrate dehydrogenase (IDH) mutations are associated with epileptic encephalopathy in patients with low-grade gliomas. Here, using electrographically sorted human cortical tissue from patients with IDH mutated tumor related epilepsy and in vitro cortical cultures, we explore a metabolic paradigm and its impact on increased neuronal excitability. We hypothesize that the IDH mutation alters metabolic pathways through its neomorphic activity of D-2-hydroxyglutarate (D-2-HG) production in turn interrupts surrounding normal neuronal circuitry potentially through metabolic perturbations. We demonstrate D-2-HG increases neuronal spiking activity, promotes distinct metabolic profiles independent of neuronal spiking activity, as well as increases neuronal mTOR signaling, which is reflected in human peritumoral epileptic cortex. Increased mTOR signaling is sufficient to upregulate neuronal spiking activity and, reciprocally, inhibition of mTOR corrects neuronal activity as well as partially corrects metabolic reprogramming. Our results suggest D-2-HG can lead to mTOR activation within the peritumoral neurons, thereby suggesting an additional possible mechanism of epileptogenesis in patients with IDH mutated low grade gliomas. Ultimately, our results raise the possibility of mTOR inhibition may be a promising treatment of seizures in patients with these tumors.

OTME-5. MENINGIOMA LIQUID BIOPSY SPECIMENS EXHIBIT CONTRASTING IMMUNE CELL LANDSCAPES ACROSS M ETHYLATION-SUBTYPES AND ESTIMATED RECURRENCE RISK SUBGROUPS

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BACKGROUND. Tumor-infiltrating immune cell compositions have been previously correlated to encouragement or inhibition of tumor growth. This association highlights immune-landscape profiling through non-invasive methods as a crucial step in approaches to treatment of patients with meningioma (MNG), a prevalent primary intracranial tumor. Genome-wide DNA methylation patterns can aid in definition and assessment of cell compositions in liquid biopsy specimens, and allow for development of machine-learning models with predictive capabilities. METHODS. We profiled the cDNA methylation (EPIC array) in liquid biopsy specimens from patients with MNG (n = 63) and nontumor controls (n = 6). We conducted both unsupervised epigenome-wide and supervised analyses of the meningioma subtypes. The immunocompetent cell composition was constructed using Python-based methodology, where a reference epigenome atlas of chosen cell types (B-cells, CD4+ and CD8+ T-cells, neutrophils, natural killer cells, monocytes, cortical neuron, vascular endothelial cells, and healthy meninge) was used to deconvolute the MNG samples. Recurrence risk was estimated using a Random-Forest classifier previously reported and validated, adapted to our serum-based cohort through employment of translatable meningioma subgroup-specific methylation markers (differentially methylated probes). RESULTS. We identified four distinct genome-wide methylation subgroups (k-clusters) of MNG which presented differential tumor micro-environments across all cell types investigated. Application of the DNA methylation-based Random-Forest classifier allowed for categorization of primary MNG serum samples to estimated recurrence-risk subgroups. Significantly contrasting micro-environments for the subgroups were observed across several cell-types, with those MNG more likely to recur displaying de

OTME-6. DEEP SEQUENCING REVEALS HETEROGENEITY OF BRAIN METASTASIS-ASSOCIATED MACROPHAGES AND MICROGLIA AND UNCOVERS THEIR CELL-TYPE-SPECIFIC FUNCTIONS WITHIN THE TUMOR MICROENVIRONMENT

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Macrophages represent a highly plastic cell type, indispensable for tissue and organ homeostasis, as well as innate immunity. Basic and translational research attributed tumor-promoting functions to macrophages, and their presence is often associated to poor patient prognosis and therapy resistance. While brain-resident macrophages, the so-called microglia (MG), represent the major immune cell type in the parenchyma, primary and metastatic brain tumors induce the recruitment of different immune cell types from the periphery, including monocyte-derived macrophages (MDM). Controversy remained about the redundancy of disease-associated molecular signatures and functions. The identification of markers that reliably distinguish brain-resident from blood-borne tumor-associated macrophages (TAMs) allowed the interrogation of molecular traits of different TAM populations in mouse and human brain tumors. Using RNA-Seq, we demonstrated that TAMs rapidly acquire disease-associated transcriptional programs upon initial tumor infiltration, while gene expression remained stable during different stages of BrM progression. Across different BrM models, disease-associated transcriptional changes revealed lineage-specific, non-redundant functions of TAM populations, which was further probed by cell-type-specific occupation of different niches within the BrM microenvironment. Furthermore, we observed dose- and cell-type-specific immune modulatory effects of whole brain radiotherapy on myeloid cells in BrM leading to a transient loss of disease-associated transcriptional programs predominately in blood-borne myeloid populations. This effect can in part at least be attributed to a replenishment of the recruited macrophage pool. This observation was further supported by scRNA-Seq analyses revealing higher heterogeneity of TAM-MDM compared to TAM-MG under treatment-naive conditions and in response to radiation treatment. Together, our results point towards the phenotypic plasticity of TAMs, especially MDMs, and the contribution of each compartment in instigating cancer-associated inflammation or the establishment of an immuno-suppressive TME. While TAMs exert functions related to pro-inflammatory responses, TAM-MDM are rather involved in tissue repair and regulation of adaptive immune cell functions.

OTME-7. CANCER - IMMUNE CELL INTERACTIONS DRIVE TRANSITIONS TO MESENCHYMAL-LIKE STATE IN GliOMA

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