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 consistent 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 clonal variations 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.

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

Artem Berezovsky[1,2], Indrani Datta[1], Ruicong She[1], Laura Hasselbach[1], Laila Poisson[1], Ana C. deCarvalho[1,2]; [1]Henry Ford Health System, Detroit, MI, USA. [2]Wayne State University, Detroit, MI, USA

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. METHODS: We have isolated ecDNA negative cell populations from two patient-derived models. HF3035 harbors a MET amplification and HF3253 harbors a PDGFRA 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 carbon 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 (R/edgeR). 2071 genes were upregulated in ecDNA+ PDX specimens and 2365 genes were downregulated. Specifically, E2F 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 PARENCHYMAL CELLS CONTROLLING NEOPLASTIC ANGIOGENESIS AND PROGRESSION

Roland Kälin[1], Linzhi Cai[1], Yuping Li[1], Louisa von Baumgarten[1], Christian Schulz[1], Ines Hellmann[2], Rainer Glass[1]; [1]University Clinics Munich, Munich, Bavaria, Germany. [2]Ludwig Maximilians University, Munich, Munich, Bavaria, Germany

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 as well as histopathology and characterized a previously unacknowledged population of 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 microglia or peripheral monocytes but were generated by a fraction of 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

Monika Dzwigonska[1], Jakub Mieczkowski[2], Paulina Pilanc[1], Salwador Cyranowski[1,3], Agata Kominek[4], Katarzyna Piwocka[4], Bozena Kaminska[1], Katarzyna B. Leszczynska[1]; [1]Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute of Experimental Biology, Warsaw, Poland. [2]Gdansk Medical University, International Research Agenda 3P, Gdansk, Poland. [3]Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland. [4]Laboratory of Cytometry, Nencki Institute of Experimental Biology, Warsaw, Poland

Chromatin structure is often dysregulated in cancers, including glioblastoma (GBM), the most common primary brain tumor in adults. GBM has the poorest prognosis and no efficient cure to date due to diffusive growth into the surrounding brain, preventing complete surgical resection and leading to inevitable tumor relapse. 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 protumorigenic 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 potently 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 genome-wide 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 hypoxia.

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

Aniello Federico[1,2], Marcel Kool[1,2]; [1]Hopp-Children's Cancer Center (KiTZ), Heidelberg, Germany. [2]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany

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-omic approach to define the TME cell populations and their contributions in the most common pediatric brain tumor entities, such as medulloblastomas and ependymomas. 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, re-clustering and marker-based cell type assignment strategies allowed to define a broad range of immune and stromal subclasses showing distinctive expression signatures reflecting variegated functional roles. By cross-matching the tumor 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