Tumor heterogeneity is one of the hallmarks of glioblastoma multiforme (GBM). Morphology within a given GBM tumor can be extremely variable where some regions of the tumor have a soft, gel-like structure while other areas are dense and fibrous. Abnormal mechanical stress and tissue stiffening caused by scarring and infiltration are believed to affect vascularity by compressing structurally weak blood vessels and restricting the supply of nutrients and oxygen to the tissue. These effects contribute to a hypoxic microenvironment that promotes disease progression and chemoresistance. The genetic and molecular mechanisms that govern tissue stiffness within GBM tumors, however, are largely unknown. Magnetic Resonance Elastography (MRE) is an emerging technique for quantifying tissue stiffness non-invasively. We have evaluated 10 GBM patients by MRE imaging obtained prior to surgical resection. During surgery, 2–7 stereotactically navigated biopsies were collected from locations within the tumor with varying degrees of measured stiffness. Biopsies were processed to extract RNA, proteins, polar metabolites and lipids. Biomolecules were analyzed on relevant -omics platforms (RNA sequencing, MS-proteomics and lipidomics, NMR of polar metabolites). Differential expression and gene set enrichment analysis of patient paired biopsies indicate an overall increase in macrophage infiltration and extracellular matrix re-organization associated with increased tumor stiffness. Among the most upregulated genes in stiff tumor tissue were lymphatic endothelial hyaluronic acid receptor 1 (LYVE-1) and macrophage receptor with collagenous structure (MARCO), both of which have been associated with immune cell infiltration and tissue stiffness. Our preliminary findings offer novel insights into tumor morphology in GBM that can be inferred from imaging prior to surgery, and can be used to identify tumor regions with high cell progression and infiltration, thereby informing and guiding surgical strategy and may ultimately lead to novel treatment strategies.

OTEH-9. SCRNA SEQUENCING OF PRONEURAL GBM AVATAR MODEL TO INFER A DEVELOPMENTAL PATH TOWARDS A GENOMICALLY UNSTABLE STATE
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Glioblastomas (GBMs) are the most common malignant primary brain tumors, and the paucity of novel treatments warrants an investigation of its origins and development into aggressive, lethal tumors. Koga & Chaim et al. have recently shown that human pluripotent stem cells (hPSCs) with different combinations of driver mutations can be differentiated into neural progenitor cells (NPCs) and engrafted into mice to form high grade gliomas (hHGGs). In this work, scRNA seq analysis was used to investigate the development of TP53-/-;PDGFRAΔ/- hHGGs, an avatar model that has been shown to recapitulate the prototypical subtype of GBM. After re-engrafting the primary avatar cultures (secondary tumor stage), the TP53-/-;PDGFRAΔ/- hHGGs developed diverse transcriptional programming and acquired a subpopulation of cells with high expression of known GBM oncogenes, such as MYC, CDK4, and PDGFRα. Notably, when all datasets were aggregated, this oncogene amplifying transcriptional program became the largest source of variation between all stages and replicates of the TP53-/-;PDGFRAΔ/- hHGGs. Indicated by a larger total copy number variation (CNV), this oncogene-amplifying program was associated with a genomically unstable developmental state. Trajectory inference could track the development of this population from the initial primary culture of TP53-/-;PDGFRAΔ/- hHGGs. Differential gene expression analysis identified distinct divergences in clonal evolution—e.g., high expression of the S100 protein family in one cluster—following the acquisition of this genomically unstable state. Later, 1st PCR was used to ascertain whether these changes in transcriptional programming were reflected in changes in DNA copy number and identified DNA amplifications of MYC and CDK4. Our scRNA seq analysis of the GBM avatar model platform provides novel insight into how oncogenic states in GBM develop from a small number of driver mutations.

OTEH-10. EVOLUTIONARY TRAJECTORY OF EPIGENOMICenoGLES OF GLOMOMADAMAS
Tathiane Malta,1 Thais Sabela,1 Indrami Datta,2,4 Frederick Yarn1, Ana Valerio Castro,3 Luciano Garrofanos2, Roel Verhaak2, Antonio Iavarone,4 Laila Poisson3,3 Houtan Noushmehr2,2 1School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil. 2Department of Neuro-Oncology at Hermelin Brain Tumor Center, Henry Ford Health System, Detroit, MI, USA. 3Department of Public Health Sciences, Henry Ford Health System, Detroit, MI, USA. 4Center for Bioinformatics, Henry Ford Health System, Detroit, MI, USA. 5The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA. 6Columbia University Medical Center, New York, NY, USA.

Gliomas are the most common malignant brain tumor, have an aggressive behavior, and invariably relapse and progress. Despite the recent advancements, little is known about the role of the epigenome in glioma disease progression and recurrence. To investigate the molecular dynamics over time and in response to therapeutic pressures, the Glioma Longitudinal AnalysisS (GLASS) Consortium, a multinational collaboration, is investigating epigenome-wide molecular changes and recurrent mutations in primary and recurrent tumor samples, including IDH mutant (IDHmut) and IDH wildtype (IDHwt) gliomas. We have compiled a total of 357 samples comprising 143 primary-recurrent pairs profiled by DNA methylation, of which 137 samples have genomic data (WXS/WGS) and 120 have transcriptomic data (RNAseq). IDHwt gliomas have a distinct epigenetic evolution compared to IDHmut after treatment. IDHwt gliomas are more epigenetically stable over time, while IDHmut gliomas display a loss of DNA methylation throughout disease progression. Next, we investigated the molecular drivers of longitudinal gliomas by integration of DNA methylation and gene expression data. We identified epigenetic activation of cell cycle pathways in recurrent IDHmut compared to initial tumors. Transcription factors mutated, ZNF637, and ZNF682 are enriched among recurrent IDHmut gliomas and potentially regulate IDHmut recurrence and progression. We next used a DNA methylation-based deconvolution approach to estimate the tumor microenvironment (TME) composition. We found that the TME among IDHmut subtypes (Codel, GCIMP-high, and GCIMP-low) presented less immune activation than IDHwt due to IDH damage regulation in IDHmut samples. Post-treatment, we found a decrease of CD4+ and an increase of CD8+ T cells in IDHmut. In conclusion, IDHwt gliomas present a more unstable epigenome, while the epigenome of IDHwt gliomas seems relatively preserved after treatment. We identified potential master regulators of cell cycle deregulation of IDHmut recurrence. Finally, the TME differs across IDHmut and IDHwt gliomas and the cell composition changes over time.

OTEH-11. SINGLE CELL RNA SEQUENCING TO IDENTIFY CELLULAR HETEROGENEITY WITHIN THE PITUITARY ADENOMAS
Saket Jais, Husam Babikut, Karin Shamardani, Aaron Diaz, Manish Aghil, University of California San Francisco, San Francisco, CA, USA.

Pituitary adenomas (PA) are one of the most common primary brain tumors and comprise approximately 15% of brain neoplasms. Most PA are...
OTHE-12. ASSESSING ADAPTIVE RESPONSES TO LOSS OF EXTRANUCLEOMORPHIC DNA AMPLIFICATION
Artem Rerezovskiy1,2, Indrani Datta1, Ruicong She1, Laura Hasselbach1, Lalita Poisson1, Ana C. de Carvalho1,2; 1Henry Ford Health System, Detroit, MI, USA; 2Wayne 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 extranucleomorph DNA segments comprised 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 responses 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 HF2353 harbors a PDGFR-constitutively active germ rearrangement and extranucleomorph amplification. We conducted paired, whole RNA-sequencing on 20 HF3253 populations (ecDNA+/-: 6 clones from 3 biological replicate PDXs) and 12 HF3035 population (ecDNA+/-: 6 clones from 3 biological replicate PDXs). RESULTS: Nonparametric differentially expressed gene (DEG) analysis using NOSeqBio (Biocoreductor), 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(-). 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, EGF targets were highly enriched in ecDNA+ populations, in addition to mRNA pre-processing. ecDNA loss primarily targeted glycogen metabolism, NTRK signaling, and mitostatin-phosphate catalysis. CONCLUSIONS: We have identified PDG-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 PARENCYMAL CELLS CONTROLLING NEOPLASTIC ANGIGENEOSE AND PROGRESSION
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Aggressive brain tumors like glioblastoma depend on support by their local environment and subsets of tumor parencymal 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 histochemical and histopathological techniques. Glioblastoma 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 monocytes but were generated from the 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 Dzwigoski1, Jakub Mieczkowski1, Paulina Pilanc1, Salvador Cyranowski1,2,1, Agata Kominek1, Katarzyna Piwacka1, Bozena Kaminska1, Katarzyna B. Leszczynska1; 1Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute of Experimental Biology, Warsaw, Poland; 2Gdansk Medical University, International Research Agenda 3P, Gdansk, Poland; 3Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland; 4Postgraduate School of Molecular Medicine, Medical University of Warsaw, 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 due to patient cure rate due to diffusion and 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 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 hypothesized 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 interrogation 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
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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-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