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

adhesion and cytokine regulation pathways. Bulk transcriptomic data of human tumors and matching patient-derived xenografts (PDXs) showed that a group of secreted stromal factors acting as regulators of tumorigenic mechanisms, such as IGF2 and COL4A1, are lost after xenografting and replaced by the host murine microenvironment, suggesting that tumor cells are involved in paracrine and bivalent crosstalk with TME cells, impacting on tumor cell growth and progression. Finally, bulk deconvolution and cell-cell communication analysis were exploited to define, respectively, the stromal cell proportions and the key factors involved in the tumor-TME crosstalk; this latter can be considered as possible targets for tailored and more specific anti-tumor therapeutic strategies.

OTME-4. IDH MUTATED GLIOMAS PROMOTE EPILEPTOGENESIS VIA D-2-HYDROXYGLUTARATE DEPENDENT MTOR HYPERACTIVATION

Armin Mortazavi[1], Islam Fayed[2], Muzna Bachani[3], Dragan Maric[1], Tyrone Dowdy[1], Mioara Larion[1], Alexander Ksendzovsky[3], Kareem Zaghloul[1]; [1]National Institute of Health, Bethesda, MD, USA. [2]Medstar Georgetown University Hospital, Washington, DC, USA. [3]University of Maryland, Baltimore, MD, USA

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 an independent factor in epileptogenesis 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 the IDH mutation promotes epileptogenesis 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 METHYLATION-SUBTYPES AND ESTIMATED RECURRENCE RISK SUBGROUPS

Grayson Herrgott, Ruicong She, Thais Sabedot, Michael Wells, Karam Asmaro, Tathiane Malta, Maritza Mosella, Kevin Nelson, Ana deCarvalho, Laila Poisson, Abir Mukherjee, Simona Cazacu, Adam Robin, Ian Lee, James Snyder, Tobias Walbert, Mark Rosenblum, Tom Mikkelsen, Steven Kalkanis, Jack Rock, Houtan Noushmehr, Ana Valeria Castro; Henry Ford Health System, Detroit, MI, USA

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 serum specimens, and allow for development of machine-learning models with predictive capabilities. METHODS. We profiled the cfDNA methylome (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 methylome. Estimation of immune cell composition was conducted using Python-based methodology, where a reference methylome atlas of chosen cell types (B-cells, CD4- and CD8T-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 an existing methylation-based 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 into 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 depletion in cell types reported to improve anti-tumoral response in many tumors (e.g. T-Cells). CONCLUSIONS. DNA methylation based deconvolution allowed for detection of contrasting tumor microenvironment compositions across MNG methylation subtypes and recurrence-risk estimation subgroups. These results suggest that microenvironment profiling can be informative of probable tumor behavior and prognostic outcomes, helping guide therapeutic approaches towards treatment of patients with MNG.

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

Michael Schulz[1,2], Tijna Alekseeva[1], Julian Anthes[1], Jandranka Macas[3,4], Birgitta Michels[1,5], Aylin Möckl[1], Katja Niesel[1], Anna Salamero-Boix[1,2], Stefan Stein[1], Henner Farin[1,5], Karl H Plate[3,4], Yvonne Reiss[3,4], Franz Rödel[6,4], Lisa Sevenich[1,4]; [1]Georg-Speyer-Haus, Frankfurt, Germany. [2]Biological Science Faculty, Goethe University, Frankfurt, Germany. [3]Institute of Neurology, University Hospital, Goethe University, Frankfurt, Germany. [4]Frankfurt Cancer Institute, Frankfurt, Germany. [5]German Cancer Consortium /German Cancer Research Center, Heidelberg, Germany. [6]Department of Radiotherapy and Oncology, Goethe University, Frankfurt, Germany

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 under normal conditions, 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 reflected 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 at least in part 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-naïve conditions and in response to radiotherapy. 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 TAM-MG 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 GLIOBLASTOMA

Toshiro Hara[1,2], Rony Chanoch-Myers[3], Nathan Mathewson[4,2], Chad Myskiw[5], Lyla Atta[6], Lillian Bussema[1], Stephen Eichhorn[7,8], Gabriela Kinker[9], Christopher Rodman[1], Nicolas Gonzalez[1,10], Hiroaki Wakimoto[11], Orit Rozenblatt-Rosen[2], Xiaowei Zhuang[7,8], Jean Fan[6], Tony Hunter[5], Inder Verma[5], Kai Wucherpfennig[2,4], Aviv Regev[2,12], Mario Suvà[1,2], Itay Tirosh[3]; [1]Department of Pathology and Center for Cancer Research, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. [2]Broad Institute of Harvard and MIT, Cambridge, MA, USA. [3]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel. [4]Department of Cancer Immunology and Virology, Department of Microbiology and Immunobiology, Department of Neurology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA. [5]Laboratory of Genetics, Salk Institute for Biological Studies, La Jolla, CA, USA. [6]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA. [7]Howard Hughes Medical Institute, Harvard University, Cambridge, MA, USA. [8]Department of Chemistry and Chemical Biology, Department of Physics, Harvard University, Cambridge, MA, USA. [9]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel. [10]Departments of Neurology and Radiation Oncology, Division of Hematology/Oncology, Massachusetts General Hospital Cancer Center, Harvard Medical School,