EPCO-04. RADIOTHERAPY IS ASSOCIATED WITH GLOBAL METHYLATION ALTERATIONS IN PATIENT DERIVED GLIOBLASTOMA CELL LINES

Aram Modrek[1], David Byun[1], Ravesanker Ezhilarasan[1], Matija Snuderl[2], and Erik Sulman[1]; [1]Department of Radiation Oncology at NYU Grossman School of Medicine, New York City, NY, USA, [2]Department of Pathology at NYU Grossman School of Medicine, New York City, NY, USA

PURPOSE/OBJECTIVE(S): In glioblastoma, DNA methylation states are the most predictive marker of overall survival and response to therapy. Our understanding of how epigenetic states, such as DNA methylation, are "mis-repaired" after DNA damage repair is scant, hampering our ability to understand how treatment associated DNA methylation alterations may drive tumor resistance and growth. MATERIALS AND METHODS: Three different patient derived IDH wild-type glioma stem cell (GSC) lines, in duplicates, were treated with radiation (20 Gray in 10 fractions vs. sham control) and allowed to recover prior to DNA methylation analysis with 850K methylation arrays. To analyze the methylation array data via bioinformatic methods we used RnBeads (version 2.4.0) and R (version 3.6.1) packages. We further focused our analysis to specific genomic regions, including CpG islands, promoters, gene bodies and CTCF motifs to understand how methylation alterations may differ between these and other genomic contexts following radiation. RESULTS: There were widespread differential methylation (pre-treatment vs. radiation treatment) changes among the genomic regions examined. Interestingly, we found differential methylation changes at CTCF motifs, which play important DNA-methylation dependent roles in gene expression and chromatin architecture regulation. Hierarchical clustering, PCA and MDS analysis of DNA methylation status amongst CpG islands, promoters, gene bodies and CTCF domains revealed strong intra-sample differences, but not inter-sample differences (between GSC lines), suggesting radiation associated methylation alterations maybe loci and context dependent. CONCLUSION: Radiation treatment is associated with wide-spread alterations of DNA methylation states in this patient derived glioblastoma model. Such alterations may drive gene expression changes or genomic architecture alterations that lead to treatment resistance, warranting further mechanistic investigation of the interplay between radiation induced DNA damage and local epigenetic state restoration following DNA damage repair.

EPCO-05. GENOME-WIDE ANALYSIS OF TEAD1 OCCUPANCY IN BIOLOGICALLY DISTINCT GLIOBLASTOMA SAMPLES

Tanvi Joshi[1], German Nudelman[2], Elena Zaslavsky[2], and Nadejda Tsankova[1]; [1]Icahn School of Medicine at Mount Sinai, New York, NY, USA, [2]Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

The diffusely infiltrative nature of glioblastoma (GBM) cells is a major contributor to the disease's aggressive behavior, including its rapid progression and therapeutic resistance. Moreover, current treatment options do not target the invasive nature of GBM. Recent chromatin accessibility studies prioritized enrichment of the TEAD1 transcription factor motif in glioblastoma stem cell biology and subsequent knockout and overexpression studies confirmed a critical role for TEAD1 in GBM migration, in vitro and in vivo. However, the downstream mechanisms through which TEAD1 regulates GBM cell migration remain poorly understood. In this study, we performed chromatin immunoprecipitation (ChIP-seq) using TEAD1-specific antibody and IgG as non-specific binding control, to characterize TEAD1 occupancy across GBM samples with unique genomic alterations. ChIP-seq peaks were called using MACS2, filtered for duplicates and blacklisted regions, and normalized per sample to their respective genomic input. Initial functional enrichment analyses were performed on three GBM samples with the highest number of TEAD1 occupancy peaks using CistromeGO, which ranked genes based on their TEAD1-specific regulatory potential (RP) score, as a function of peak number and distance from the transcription start site. Analyses of the top 1000 genes with highest TEAD1 RP scores identified 132 common targets across all samples, including known TEAD target genes *ETV1* and *Cyr61*, which related to angiogenesis, cadherin and integrin signaling, cell adhesion, and chromatin regulation gene ontology terms, among others. Interestingly, KEGG pathway analysis also revealed Hippo pathway enrichment across all GBM samples, suggesting a possible TEAD1 regulatory feedback loop in GBM. Analysis of TEAD1 ChIP-seq peaks in non-GBM negative control tissue did not show functional enrichment for any of the terms seen in the GBM samples. Ongoing analyses are focused on characterizing TEAD1 occupancy at active cis-regulatory regions using parallel H3K27ac ChIP-seq data, in order to prioritize the most salient TEAD1-regulatory targets in GBM.

EPCO-06. AGE- AND REGION-SPECIFIC MULTI-OMIC CHARACTERIZATION OF H3-K27M MUTANT DIFFUSE MIDLINE GLIOMA

Ilon Liu[1], Jiang Li[2], Daeun Jeong[3], Olivia A. Hack[3], McKenzie Shaw[3], Bernhard Englinger[3], Byron Avihai[3], Kati Ernst[4], Adam Resnick[5], Aaron Diaz[6], David Jones[4], Carl Koschmann[7], Claudia Kleinman[8], Nada Jabado[8], Jennifer Cotter[9], Keith Ligon[1], Sanda Alexandrescu[10], WK Alfred Yung[11], Thomas Czech[12], Isabel Arrillaga-Romany[13], Johannes Gojo[14], Irene Slavc[15], Michelle Monje[16], and Mariella G. Filbin[17]; [1]Dana-Farber Cancer Institute, Boston, USA, [2]Dana-Farber Boston Children's Cancer, Bostan, USA, [3]Dana-Farber Boston Children's Cancer and Blood Disorders Center, Boston, MA, USA, [4]Hopp Children's Cancer Center Heidelberg (KiTZ) and Pediatric Glioma Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany, [5]Center for Data Driven Discovery in Biomedicine, Children's Hospital of Philadelphia, Philadelphia, USA, [6]University of California, San Francisco, San Francisco, CA, USA, [7]University of Michigan, Ann Arbor, MI, USA, [8]Department of Human Genetics, McGill University, Montreal, Canada, [9]Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, Keck School of Medicine of University of Southern California, Los Angeles, USA, [10]Department of Pathology, Boston Children's Hospital, Boston, MA, USA, [11]University of Texas, MD Anderson Cancer Center, Houston, TX, USA, [12]Department of Neurosurgery, Medical University of Vienna, Vienna, Wien, Austria, [13]Massachusetts General Hospital, Boston, ME, USA, [14]Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria, [15]Medical University of Vienna - Department of Pediatrics and Adolescent Medicine, Vienna, Austria, [16]Stanford University, Stanford, CA, USA, [1 7]Department of Pediatric Oncology, Dana-Farber Boston Children's Cancer and Blood Disorders Center, Boston, USA

Diffuse midline gliomas driven by lysine27-to-methionine mutations in histone 3 (H3-K27M DMGs) are among the most fatal brain tumors. Molecular studies including single cell RNA-sequencing (scRNA-seq) of pediatric and predominantly pontine H3-K27M DMGs have shown that the H3-K27M oncohistone keeps glioma cells locked in a stem-like oligodendrocyte precursor cell (OPC) state that is capable of self-renewal and tumor-initiation. However, a comprehensive dissection of the cellular architecture of H3-K27M DMGs across different midline regions and age groups is required to better understand the cell-intrinsic and contextual regulation of H3-K27M DMG cell identities. In particular, the more recently described group of adult H3-K27M DMGs remains understudied.

Here, we have collected and characterized 45 H3-K27M mutant patient tumors, spanning pontine (n=26), thalamic (n=17), and spinal (n=2) locations. Median age at surgery was 12 (2-68) years, encompassing 21 early childhood (0-10 years), 12 adolescent (11-20 years), and 12 adult (≥ 21 years) tumors. The majority of samples were obtained pre-treatment (n=28), as opposed to post-treatment or at autopsy (n=17). We profiled all 45 tumors by single cell/single nucleus RNA-seq and selected tumors were further characterized by the single cell assay for transposase-accessible chromatin (scATAC-seq). Our integrated analyses highlight the predominance of transcriptionally and epigenetically defined OPC-like tumor cells as the main cell population of H3-K27M DMGs across all age groups and locations. We further identify distinct age- and location-specific OPC-like cell subpopulations. Comparison of pediatric and adult tumors further demonstrates a significant increase of mesenchymal cell states in adult H3-K27M DMGs, which we link to differences in glioma-associated immune cell compartments between age groups. Together, this study sheds light on the effects of age- and region-dependent microenvironments in shaping cellular identities in H3-K27M DMGs.

EPCO-07. HYBRID NEURO-GLIAL CELLULAR ARCHITECTURE IN HIGH-GRADE GLIOMA DRIVEN BY H3-G34R MUTATION

Julie Laffy[1], Masashi Nomura[2], Chen He[3], Lillian Bussema[2], Michal Slyper[4], Laura Hover[3], Simon Gritsch[2], Aviv Regev[4], Suzanne Baker[3], Itay Tirosh[1], and Mario Suva[2]; [1]Weizmann Institute of Science, TEL AVIV - YAFO, Israel, [2]Massachussets General Hospital, Boston, MA, USA, [3]St. Jude Children's Research Hospital, Memphis, TN, USA, [4]Broad Institute of Harvard and MIT, Boston, MA, USA

High-grade gliomas (HGG) with histone H3.3 G34R mutation are rare intractable tumours in the cerebral hemispheres that preferentially affect adolescents and young adults, but have unknown mechanisms of neuroanatomical specificity and tumourigenesis. Here, we performed single-nucleus RNA-sequencing of twenty patient samples, encompassing twelve tumours with G34R mutation and eight H3.3 wildtype HGGs, age- and location-matched. Both classes of HGG were heterogeneous, with malignant cells in multiple states, recapitulating neural and glial developmental trajectories. G34R HGG is distinguished by lack of malignant cells in the oligodendroglial lineage, and aberrant expression of neuronal programs superimposed over cellular states, resulting in hybrid glio-neuronal malignant programs. Singe-cell barcoding supports plasticity between cellular states in HGG with multiple possible transitions. CRISPR-correction of G34R in HGG models followed by scRNA-seq supports that the G34R mutation directly drives these aberrant programs. Our study provides a framework for studying the origin and tumourigenesis of paediatric gliomas.