OPTC-5. MOLECULAR SIGNATURES OF PODOPLANIN EXPRESSING GLIOBLASTOMA CELL SUBSETS WITH PUTATIVE ROLE IN CANCER ASSOCIATED THROMBOSIS AND MICROTHROMBOSIS

Nadim Tawil[1,2], Rayhaan Bassawon[1], Brian Meehan[2], Laura Montermini[2], Dongsic Choi[2], Ali Nehme[1,3], Hamed Najafabadi[1,3], Yasser Riazalhosseini[1,3], Nicolas De Jay[4], Claudia Kleinman[4], Tenzin Gayden[1], Cristiana Spinelli[1,2], Lata Adnani[2], Charles Couturier[5], Kevin Petrecca[5], Nada Jabado[1,2], Janusz Rak[1,2]; [1]McGill University, Montreal, QC, Canada. [2]Research Institute of McGill University Health Center, Montreal, QC, Canada. [3]McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada. [4]Lady Davis Research Institute - Jewish General Hospital, Montreal, QC, Canada. [5]Montreal Neurological Institute and Hospital, Montreal, QC, Canada

Vascular anomalies, including thrombosis, are a hallmark of glioblastoma (GBM) and an aftermath of dysregulated cancer cell genome and epigenome. Upregulation of podoplanin (PDPN) by cancer cells has recently been linked to an increased risk of venous thromboembolism in glioblastoma patients. Thus, regulation of this platelet activating transmembrane protein by transforming events and release from cancer cells into the circulation are of considerable interest. We took advantage of single-cell and bulk GBM transcriptome dataset mining and investigated the pattern of PDPN expression across several databases. Our analysis indicated that PDPN is expressed by distinct (mesenchymal) glioblastoma cell subpopulations and is downregulated by oncogenic mutations of EGFR and IDH1 genes, via changes in chromatin modifications (EZH2) and DNA methylation, respectively. Additionally, we utilized isogenic and stem GBM cell lines, xenograft models in mice, ELISA assays for PDPN, tissue factor (TF), platelet factor 4 (PF4) and clotting activation markers (D-dimer), and multicolor nano-flow cytometry to show that GBM cells exteriorize PDPN and/or TF as cargo of exosome-like coagulant extracellular vesicles EVs. We also documented an increase of platelet activation (PF4) or coagulation markers (D-dimer) in mice harboring the corresponding PDPN- or TF-expressing glioma xenografts, respectively. While PDPN was a dominant regulator of systemic platelet activation, co-expression of PDPN and TF impacted local microthrombosis. Our work suggests that distinct cellular subsets drive multiple facets of GBM-associated thrombosis and may represent targets for diagnosis and intervention.

FINAL CATEGORY: OMICS OF RESPONSE TO THERAPY

OMRT-1. CANNABIDIOL CONVERTS NFKB INTO A TUMOR-SUPPRESSOR IN GLIOBLASTOMA WITH DEFINED ANTIOXIDATIVE PROPERTIES

Marie Volmar[1], Jiying Cheng[1], Michael Synowitz[2], Joel Schick[3], Roland Kälin[1], Rainer Glass[1]; [1]University Clinics Munich, Munich, Bavaria, Germany. [2]University Clinics Kiel, Kiel, Schleswig-Holstein, Germany. [3]Helmholtz Center Munich, Munich, Bavaria, Germany

BACKGROUND: The transcription factor NFKB drives neoplastic progression of many cancers including primary brain tumors (glioblastoma; GBM). Precise therapeutic modulation of NFKB-activity can suppress central oncogenic signalling pathways in GBM, but clinically applicable compounds to achieve this goal have remained elusive. METHODS: In a pharmacogenomics study with a panel of transgenic glioma cells we observed that NFKB can be converted into a tumor-suppressor by the non-psychotropic cannabinoid Cannabidiol (CBD). Subsequently, we investigated the anti-tumor effects of CBD, which is used as an anticonvulsive drug (Epidiolex) in pediatric neurology, in a larger set of human primary GBM stem-like cells (hGSC). For this study we performed pharmacological assays, gene-expression profiling, biochemical and cell-biological experiments. We validated our findings using orthotopic in vivo models and bioinformatics-analysis of human GBM-datasets. RESULTS: We found that CBD promotes DNA-binding of the NFKB-subunit RELA and simultaneously prevents RELA-phosphorylation on serine-311, a key residue which permits genetic transactivation. Strikingly, sustained DNA-binding by RELA lacking phospho-serine 311 was found to mediate hGSC-cytotoxicity. Widespread sensitivity to CBD was observed in a cohort of hGSC defined by low levels of reactive oxygen-species (ROS), while high ROS-content in other tumors blocked CBD induced hGSC-death. Consequently, ROS-levels served as predictive biomarker for CBD-sensitive tumors. CONCLUSIONS: This evidence demonstrates how a clinically approved drug can convert NFKB into a tumor-suppressor and suggests a promising repurposing option for GBM-therapy.

OMRT-2. LIQUID BIOPSY FOR PATIENT STRATIFICATION AND MONITORING OF DACOMITINIB CLINICAL TRIAL IN PATIENTS WITH EGFR AMPLIFIED RECURRENT GLIOBLASTOMA

Anudeep Yekula[1], Robert Kitchen[1], Sudipto Chakrabortty[2], Bob Carter[1], Xandra Breakefield[1], Johan Skog[1], Leonora Balaj[1]; [1]Massachusetts General Hospital, Boston, MA, USA. [2]Exosome Diagnostics, Waltham, MA, USA

INTRODUCTION: Liquid biopsy for the detection and monitoring of brain tumors is of significant clinical interest. The ability to non-invasively profile tumors can avoid a risky biopsy and opens avenues for testing novel therapies by accurately stratifying patients to receive the right therapy. Here, we provide evidence of EV RNA-based diagnosis, patient stratification, and assessment of response to therapy in the setting of a clinical trial evaluating the efficacy of dacomitinib, an EGFR tyrosine kinase inhibitor in patients with recurrent, EGFR amplified GBM(NCT01112527). METHODS: We performed RNASeq on long RNA extracted from the serum samples, pre-treatment and 1-month post-treatment. RESULTS: Firstly, longRNASeq allowed the detection of thousands of mRNA, lincRNAs and antisense RNAs enabling the study of a wider repertoire of potential RNA based biomarkers. Secondly, we observed a differential expression profile in serum EV RNA of GBM patients and healthy controls. Combining our findings with TCGA data and literature screening, we generated a 25 gene signature representative of critical pathways in several hallmarks of cancer. Thirdly, we observed a differential expression profile in serum EV RNA of responders to dacomitinib compared to non-responders in pre-treatment serum. Specifically, the EV mRNAs ZNF35 and LAMTOR2 distinguish responders from non-responders (p-adjusted = 2.6E-8 and 2.4E-6, respectively) allowing potential patient stratification. Finally, we observed a differential expression profile in serum EV RNA of responders to dacomitinib compared to non-responders in post-treatment serum. EV mRNA DNMT3A is significantly enriched (p-adjusted = 1.8E-4) in post-treatment serum of responders compared to non-responders to dacomitinib allowing potential monitoring of response to therapy. CONCLUSION: This study represents the first longitudinal profiling of the EV transcriptome in a cohort of genomically selected GBM patients. These findings are a tantalizing step toward liquid biopsy-based biomarkers for the detection of GBM, as well as patient stratification and monitoring.

OMRT-3. LONGITUDINAL ANALYSIS OF DIFFUSE GLIOMA REVEALS CELL STATE DYNAMICS AT RECURRENCE ASSOCIATED WITH CHANGES IN GENETICS AND THE MICROENVIRONMENT

Frederick Varn[1], Kevin Johnson[1], Floris Barthel[1], Hoon Kim[1], Taylor Wade[1], Tathiane Malta[2], Thais Sabedot[3], Disha Lodha[4], Shoaib Ajaib[4], Nazia Ahmed[4], Luciano Garofano[5], Fulvio D'Angelo[5], Lucy Stead[4], Laila Poisson[6], Houtan Noushmehr[3], Antonio Iavarone[5,7], Roel Verhaak[1], GLASS Consortium[1]; [1]The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA. [2]University of Sao Paulo, Ribeirao Preto, Brazil. [3]Department of Neurosurgery, Henry Ford Health System, Henry Ford Cancer Institute, Detroit, MI, USA. [4]Leeds Institute of Medical Research at St James's, University of Leeds, Leeds, UK. [5]Institute for Cancer Genetics, Columbia University Medical Center, New York, NY, USA. [6]Department of Biostatistics, Henry Ford Health System, Henry Ford Cancer Institute, Detroit, MI, USA. [7]Department of Neurology, Columbia University Medical Center, New York, NY, USA

Diffuse glioma is an aggressive brain cancer that is characterized by a poor prognosis and a universal resistance to therapy. The evolutionary processes behind this resistance remain unclear. Previous studies by the Glioma Longitudinal Analysis (GLASS) Consortium have indicated that therapy-induced selective pressures shape the genetic evolution of glioma in a stochastic manner. However, single-cell studies have revealed that malignant glioma cells are highly plastic and transition their cell state in response to diverse challenges, including changes in the microenvironment and the administration of standard-of-care therapy. Interactions between these factors remain poorly understood, making it difficult to predict how a patient's tumor will evolve from diagnosis to recurrence. To interrogate the factors driving therapy resistance in diffuse glioma, we collected and analyzed RNA- and/or DNA-sequencing data from temporally separated tumor pairs of 292 adult patients with IDH-wild-type or IDH-mutant glioma. Recurrent tumors exhibited diverse changes that were attributable to changes in anatomic composition, somatic alterations, and microenvironment interactions. Hypermutation and acquired *CDKN2A* homozygous deletions associated with an increase in proliferating stem-like malignant cells at recurrence in both glioma subtypes, reflecting active tumor expansion. IDH-wild-type tumors were more invasive at recurrence, and their malignant cells exhibited increased expression of neuronal signaling programs that reflected a possible role for neuronal interactions in promoting glioma progression. Mesenchymal transition was associated with the presence of a specific myeloid cell state defined by unique ligand-receptor interactions with malignant cells, providing opportunities to target this transition through therapy. Collectively, our results uncover