CBMT-23. NON-CANONICAL FUNCTIONS OF TERT IN GLIOBLASTOMA

Shayesteh Ferdosi[1], Brett Taylor[1], Saumya Bollam[2], Vijay Gokhale[3], Laurence Hurley[3], Harshil Dhruv[1], and Michael Berens[1]; [1]Translational Genomics Research Institute, Phoenix, AZ, USA, [2]Translational Genomics Research Institute, San Francisco, CA, USA, [3]University of Arizona, Tucson, AZ, USA

Telomerase is an enzyme with a catalytic subunit, telomerase reverse transcriptase (TERT), that is in charge of telomere elongation in the nucleus. Promotor region of TERT is commonly mutated across cancers, especially in glioblastoma (GBM) with over 80% frequency. In the absence of any effective molecular targeting therapy for GBM, elucidating oncogenic signaling of TERT could open new avenues in GBM treatment. Canonically, mutations of TERT, which result in TERT upregulation, maintain telomere length in the nucleus and promote indefinite proliferation of cancer cells. However, a non-canonical function of TERT in the mitochondria has recently been suggested. We screened GBM cell models against a novel small molecule inhibitor (RG1534, Reglagene Inc.) that interferes with the functionality of a mutated hTERT promoter. RG1534 selectively suppresses glioma cell viability without affecting non-transformed normal human astrocytes. More interestingly, RG1534 treatment leads to rapid apoptosis induction in glioma cell lines that does not correlate with the time course of the telomere shortening effect. We further validated this rapid apoptosis behavior in glioma cell lines using siRNA and CRISPR/Cas-9 mediated hTERT knockdown. We also measured the protein expression of TERT in subcellular fractions of glioma cell lines and demonstrated the presence of higher TERT expression in mitochondrial extract compared to the nucleus. Finally, using MitoSOX dye we assessed ROS generation in glioma cells in response to an oxidant with or without TERT expresssion. In summary, our results demonstrate that non-canonical functions of TERT may play critical role in glioma pathobiology and require more detail investigation.

CBMT-24. CHARACTERIZATION OF PRIMARY CILIUM IN RECURRENT GLIOBLASTOMA: IMPLICATIONS FOR NEW THERAPEUTIC TARGETS

Vittorio Stumpo[1], Aruljothi Marappian[2], Quintino Giorgio D'Alessandris[1], Simone Pacioni[1], Rina Di Bonaventura[1], Lara Ebbert[2], Martina Giordano[1], Valerio Caccavella[1], Alessandro Olivi[3], Maria Laura Falchetti[4], Lucia Ricci-Vitiani[5], Roberto Pallini[1], and Jay Gopalakrishnan[2]; [1]Department of Neurosurgery, Fondazione Policlinico Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy, [2]Heinrich-Heine-Universität Düsseldorf, Dusseldorf, Germany, [3]Catholic University - Rome, Rome, Italy, [4]Institute for Cell Biology and Neurobiology, National Research Council, Rome, Italy, [5]Department of Oncology and Molecular Medicine, Italian National Health Institute, Rome, Italy

INTRODUCTION: Primary cilium is a highly conserved, dynamic cellular organelle which plays several roles in embryonic development, intracellular signaling, and cell cycle. Structural alterations of primary cilium have been described in human gliomas including glioblastoma (GBM), however, its actual role in pathogenesis and treatment resistance of these tumors is largely unknown. METHODS: We investigated cilium morphology and expression of cilium-related genes in human glioma of various WHO grade and in couples of patient-derived glioma stem-like cells (GSCs) that were established from the very same GBM at first diagnosis and at recurrence. Immunohistochemistry with anti-Arl13b antibody was used to assess cilium morphology. The expression levels of genes involved in ciliary disassembly complex (CDC) were analyzed by quantitative real-time PCR, using neural progenitor cells (NPCs) as control. Lastly, we assessed 3 GSC cultures that were treated with a drug inhibiting cilia disassembly (CCB-Cil). RESULTS: Anaplastic oligodendroglioma and proneural GBM showed the highest percentage of ciliated cells. In GBM, we found the highest percentage of fragmented cilia. GSCs derived from newly diagnosed GBMs displayed lower percentages of ciliated cells than those derived from recurrent GBMs (20% vs 70%). Morphological analysis indicated that GSCs from recurrent GBM show cilia with extremely various morphology compared with GSCs from newly diagnosed GBM and NPCs. Gene analysis showed reduced expression of CDC-related genes in GSCs from newly diagnosed GBM with respect to those from recurrent GBMs. CCB-Cil treatment determined a global reduction of CDC-related genes, increased expression of differentiation markers (GFAP), and reduction of stemness markers (SOX2). CONCLUSIONS: The increased percentage of ciliated cells in GSCs from recurrent GBM may be related to a compensatory response of CDC and to an accelerated ciliary turnover. Blocking cilia disassembly reduces stemness features and induces differentiation in GSCs, suggesting that this approach could represent a promising strategy for targeting GBM.

CBMT-25. THE KLF4^{K409Q} MUTATION IN MENINGIOMA IMPAIRS HIF-1A DEGRADATION AND CAN BE HARNESSED FOR TARGETED THERAPY

Niklas von Spreckelsen[1], Natalie Waldt[2], Rebecca Poetschke[3], Christoph Kesseler[2], Hildegard Dohmen[4], Hui-Ke Jiao[4], Attila Nemeth[4], Martina Deckert[5], Frank Angenstein[6], Boris Krischek[7], Pantelis Stavrinou[7], Marco Timmer[7], Marc Remke[8], Elmar Kirches[9], Roland Goldbrunner[10], Ennio Chiocca[1], Stefan Huettelmaier[3], Till Acker[4], and Christian Mawrin[2]; [1]Department of Neurosurgery, Brigham and Women's Hospital | Harvard Medical School, Boston, MA, USA, [2]Department of Neuropathology, Otto-von-Guericke University, Magdeburg, Magdeburg, Sachsen-Anhalt, Germany, [3]Institute of Molecular Medicine, Martin Luther University, Halle/Saale, Halle/Saale, Germany, [4]Department of Neuropathology, University Giessen, Giessen, Germany, [5]Department of Neuropathology, University Hospital Cologne, Cologne, Germany, [6]Laboratory for Non-invasive Imaging, Magdeburg, Magdeburg, Germany, [7]Department of Neurosurgery, Center for Neurosurgery, Faculty of Medicine and University Hospital, University of Cologne, Cologne, Germany, [8]Department of Pediatric Oncology, Hematology and Clinical Immunology German Cancer Consortium (DKTK), University Hospital Düsseldorf, Duesseldorf, Germany, [9]Department of Neuropathology, Otto-von-Guericke University, Magdeburg, Magdeburg, Germany, [10]Dept. of General Neurosurgery, University of Cologne, Cologne, Germany

Recently, several Non-*NF2* driver mutations (*KLF4, TRAF7, SMO, AKT1*^{E17K}) in meningioma have been identified. While they have been shown to correlate with certain pathological subtypes and locations, the clinical impact and repercussions on cellular pathways have largely remained elusive. Through analysis of clinical, pathological and preoperative imaging data of 96 patients and sequencing of the corresponding 96 tumor samples for the *Krueppel like factor 4-K409Q* mutation (*KLF4*^{K409Q}) we present evidence that the *KLF4*^{K409Q} tumors harbour an increased risk for peritumoral brain edema (PTBE) and can be predicted with the edema-index, a simple tool based on preoperative imaging. Further analysis involving RNA-sequencing of a matched subset of 7 *KLF4*^{K409Q} and 10 *KLF4-wildtype* (*wt*) tumors revealed a significant shift of gene expression and the upregulation of hypoxia driven pathways, including VEGF levels, in *KLF4*^{K409Q} tumors. On the cellular level, we go on to show that the *KLF4*^{K409Q} mutation results in an increased KLF-4 stability as well as the inhibition of hydroxylation dependent degradation of HIF1-α and a significant increase of VEGF expression under hypoxic conditions. Finally, we demonstrate that this upregulation of VEGF in *KLF4*^{K409Q} cells can be inhibited by targeting the mammalian target of rapamycin (mTor) with Temsirolimus. In summary we show that the *KLF4*^{K409Q} mutation in meningioma has highly relevant repercussions in both, the biological and clinical context and can be harnessed for targeted therapy.

CBMT-26. SERIAL CHARACTERIZATION OF HYPERPOLARIZED [1-^{13}C]PYRUVATE METABOLISM IN PATIENTS WITH GLIOMA AND THE INFLUENCE OF BEVACIZUMAB

Adam Autry, Jeremy Gordon, Hsin-Yu Chen, Marisa Lafontaine, Javier Villanueva-Meyer, Susan Chang, Duan Xu, Peder Larson, Daniel Vigneron, and Yan Li; University of California, San Francisco, San Francisco, CA, USA

Treatment-related changes often mimic or mask tumor on standard anatomic imaging, making it difficult to monitor disease recurrence. Hyperpolarized (HP) carbon-13 MR imaging allows for real-time non-invasive measurement of metabolism, which may improve patient surveillance. Here, we focused on characterizing serial HP scans in patients undergoing treatment compared to healthy controls. Serial dynamic HP C-13 MRI scans were performed on 5 patients with recurrent glioma (22 total) and 3 healthy controls (4 total) using an echo-planar imaging sequence (2.88-8cm^3 spatial resolution, 3s temporal resolution, 60s), following injection of 0.43mL/kg of 250mM HP [1-^{13}C]pyruvate. Apparent rate constants were modeled for enzymatic conversion of pyruvate-to-lactate (k_{PL}) via cytosolic lactate dehydrogenase and pyruvate-to-bicarbonate (k_{PB}) via mitochondrial pyruvate dehydrogenase and carbonic anhydrase. Regions of interest included normal-appearing white matter (NAWM) and T2-hyperintense lesions (T2L), which were segmented from H-1 MR images and then aligned to the HP data. Carbon voxels containing >30% of NAWM or T2L were included in the analysis. Healthy controls demonstrated consistent k_{PL} and k_{PB} values over 4 scans in NAWM with SD/Mean of 5% and 12%, respectively. Compared to the median $k_{PL\text{-}NAWM}$ of 0.022s^{-1} in controls, the 5 patients had median serial $k_{PL\text{-}NAWM}$ values of 0.023, 0.023, 0.023, 0.029, and 0.015s^{-1}, and mean serial ratios of k_{PL} between T2L and NAWM ($k_{PL\text{-}T2L}$/$k_{PL\text{-}NAWM}$) of 1.22, 1.27, 1.05, 1.32, and 1.37s^{-1}, indicating higher values in putative tumor. Median $k_{PB\text{-}NAWM}$ in controls was 0.004s^{-1} and ranged in patients 0.003-0.006s^{-1}. Two patients with >4 serial scans, showed consistent $k_{PL\text{-}NAWM}$ over standard-of-care treatment and elevated $k_{PL\text{-}T2L}$ within new lesions, but up to 85% increase in $k_{PL\text{-}NAWM}$ with bevacizumab, which may be attributed to reduced BBB permeability. Stable patients generally demon-