Fecal samples were collected from humans with newly diagnosed glioma before resection, chemoradiation, and after chemoradiation (16s RNA, metabolomic, neurotransmitter analysis). In mice, FM beta diversity was significantly altered with glioma (p=0.003) while the alpha diversity remained unchanged. At a genus and family level analysis the relative abundance of Bacteroides (p=0.01) and Bacteroidaceae (p=0.02) was increased. Beta diversity of mice receiving 5mg/kg TMZ changed from baseline (p=0.02). Collectively, this suggests that glioma alters the FM, to what consequence remains to be explored. Alpha (Observed OTUs, p=0.029) and beta diversity (p=0.034) differences in mice correlated with survival (< 25 - >25 days). In humans, norepinephrine and 5-hydroxyindoleacetic acid were significantly lower in glioma patients at diagnosis compared to controls. Our findings demonstrate for the first time the relationship between glioma and the gut-brain axis. Understanding alterations in the FM in glioma patients may allow novel interventions and should be further investigated.

CBMT-41. IMAGING A HALLMARK OF CANCER: HYPERPOLARIZED ^{13}C-MAGNETIC RESONANCE SPECTROSCOPY CAN NON-INVASIVELY MONITOR TERT EXPRESSION IN LOW-GRADE GLIOMAS IN VIVO

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Telomerase reverse transcriptase (TERT) expression is a hallmark of cancer, including in primary glioblastomas and low-grade oligodendrogliomas. Since TERT is essential for glioma proliferation and is an attractive therapeutic target, metabolic imaging of TERT status can inform on tumor progression and response to therapy. To that end, the goal of this study was to identify non-invasive, translational, hyperpolarized ^{13}C-magnetic resonance spectroscopy-detectable metabolic imaging biomarkers of TERT in low-grade oligodendrogliomas. Unbiased metabolomic analysis of immortalized normal human astrocytes without ($NHA_{control}$) and with TERT (NHA_{tert}) indicated that TERT induced unique metabolic reprogramming. Notably, TERT increased NADPH and NADH levels. Glucose flux through the pentose phosphate pathway (PPP) is a major producer of NADPH. Non-invasive imaging of PPP flux using hyperpolarized [U-^{13}C,U-^{2}H]-glucose indicated that production of the PPP metabolite 6-phosphogluconate (6-PG) was elevated in NHA_{tert} cells relative to $NHA_{control}$. Importantly, hyperpolarized [U-^{13}C,U-^{2}H]-glucose flux to 6-PG clearly differentiated tumor from normal brain in orthotopic NHA_{tert} tumor xenografts. Next, we exploited the observation that TERT expression increased NADH, which is essential for the metabolism of hyperpolarized [1-^{13}C]-alanine to lactate. Lactate production from hyperpolarized [1-^{13}C]-alanine was higher in NHA_{tert} cells relative to $NHA_{control}$. Importantly, hyperpolarized [1-^{13}C]-alanine imaging in orthotopic NHA_{tert} tumors revealed pronounced differences in lactate production between tumor tissue and normal brain. Mechanistically, TERT increased expression of glucose-6-phosphate dehydrogenase (G6PDH), the rate-limiting enzyme for 6-PG and NADPH production, and of nicotinamide phosphoribosyltransferase (NAMPT), a rate-limiting enzyme for NADH biosynthesis. Silencing TERT reversed G6PDH and NAMPT expression and normalized hyperpolarized [U-^{13}C,U-^{2}H]-glucose and [1-^{13}C]-alanine metabolism, validating our imaging biomarkers. Finally, hyperpolarized [U-^{13}C,U-^{2}H]-glucose and [1-^{13}C]-alanine could monitor TERT status in the clinically relevant, patient-derived BT54 oligodendroglioma model. In summary, we demonstrate, for the first time, non-invasive *in vivo* imaging of TERT status in gliomas that can enable longitudinal analysis of tumor burden and treatment response in the clinic.

CBMT-42. A GENOME-WIDE CRISPR-Cas9 SCREEN FOR GENES REGULATING QUIESCENT-LIKE STATES IN GLIOBLASTOMA

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Current standard of care therapy for glioblastoma (GBM) includes cytoreduction followed by ablative therapies that target rapidly dividing cell types. However, non-cycling, quiescent-like states (G0 phase cells) are present in both normal tissue and tumors and play important roles in maintaining heterogeneity and cellular hierarchies. The presence of quiescent-like/G0 states therefore represents a natural reservoir of tumor cells that are resistant to current treatments. Quiescence or G0 phase is a reversible state of "stasis" cells enter in response to developmental or environmental cues. However, it remains largely unclear to what degree or by what mechanisms tumor cells enter into or exit from quiescent-like states. To gain insight into how glioblastoma cells might regulate G0-like states, we performed a genome-wide CRISPR-Cas9 screen in patient-derived GBM stem-like cells (GSCs) harboring a p27-mVenus reporter construct, which is stabilized when cells enter a G0-like state. By assaying p27 reporteractivity, we were able to identify sgRNAs enriched in $p27^{hi}$populations and, which upon retest, trigger a G0-like arrest in GSCs. Among the top screen hits were members of the Tip60/KAT5 histone acetyltransferase complex, including KAT5 itself. Remarkably, we show that downregulation of KAT5 in vitro and in vivo dramatically increases the pool of cells in G0-like states in GSC cultures and GSC-induced tumors. Using single cell RNA-sequencing, we show that this cell state is characterized by gene expression signatures similar to those found in non-dividing subpopulations of GBM tumors and quiescent neural stem cells. In addition, we perform in-depth molecular and phenotypic characterization of these induced G0-like states, including epigenetic and metabolic profiles. These suggest a key role for KAT5 in regulating genes related to protein synthesis. In summary, our results suggest that Tip60/KAT5 activity plays key roles in G0 ingress/egress for GBM tumors and may provide novel therapeutic opportunities.

CBMT-43. INTEGRATED LIPIDOMIC AND MOLECULAR ANALYSIS REVEALS A SUBSET OF GLIOBLASTOMA VULNERABLE TO FERROPTOSIS

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Cancers, including the universally lethal glioblastoma (GBM), have reprogrammed lipid metabolism to fuel tumor growth. However, the molecular alterations responsible for aberrant lipid metabolism, and the potential for identifying new therapeutic opportunities are not fully understood. To systematically investigate the GBM lipidome, we performed integrated transcriptomic, genomic and shotgun lipidomic analysis of a library of molecularly diverse patient-derived GBM cells (*n=30*). Using this comprehensive approach, we discovered two GBM sub-groups defined by their combined molecular and lipidomic profile. Polyunsaturated fatty acids (PUFAs) were among the most significant lipids that distinguished these two groups of GBM tumors. Intriguingly, this lipid metabolic phenotype was associated with heightened sensitivity to ferroptosis – a newly discovered form of regulated cell death. As PUFA oxidation is a critical feature of ferroptosis, our findings suggest a novel association between specific molecular signatures of GBM, lipid metabolism and ferroptosis. This relationship may present a new therapeutic opportunity to target ferroptosis in a molecularly-defined subset of GBMs.

CBMT-44. COMPREHENSIVE CHARACTERIZATION OF THE INTRINSIC APOPTOTIC MACHINERY REVEALS THE MOLECULAR BLOCKS RESPONSIBLE FOR RESISTANCE TO CELL DEATH IN GLIOBLASTOMA

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Conventional therapies (e.g., temozolomide (TMZ), Irradiation (IR)) transiently halt tumor growth of glioblastoma (GBM) but fail to induce cell death through apoptosis. Consequently, the inability to kill GBM tumor cells ultimately leads to disease progression and a poor patient survival. The precise molecular mechanisms by which GBM are refractory to apoptosis remain enigmatic. We preformed BH3 profiling to functionally characterize the intrinsic apoptotic machinery and define the molecular 'blocks' that obstruct GBM apoptosis under both basal and treatment states. Using a molecularly diverse panel of freshly purified patient tumors, patient-derived neurospheres and patient-derived orthotopic xenografts, we identified that nearly all GBMs have two anti-apoptotic blocks, BCL-xL and MCL-1, which are essential for GBM survival in an untreated state. TMZ or IR (TMZ/IR) disabled the MCL-1 block in a subset of GBMs, leaving tumors exclusively dependent on BCL-xL for survival. Mechanistic studies revealed that TMZ/IR treatment induced p53-dependent expression of the pro-apoptotic protein, PUMA, which subsequently bound to and neutralized MCL-1. Consequently, pharmacological inhibition of BCL-xL in combination with TMZ/IR initiated intrinsic apoptosis and was synergistically lethal in p53 wild-type GBM. These studies identify the existence of two anti-apoptotic proteins that are critical for GBM survival, which can be therapeutically exploited in a molecularly defined subset of GBMs for tumor eradication.

CBMT-45. SEX-SPECIFIC METABOLIC ADAPTIONS IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and aggressive brain tumor in adults. GBM occurs more commonly in males, but female patients sur-