Title

METB-14. DOWN-REGULATION OF ACETATE METABOLISM TOWARDS FATTY ACIDS IN IDH1 MUTANT GLIOMA

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METB-12. METABOLIC RE-PROGRAMING MEDIATES BIDIRECTIONAL SHIFT BETWEEN TRANSCRIPTIONAL SUBCLASSES AND DRIVES TUMOR HETEROGENEITY

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The highly heterogeneous nature of malignant brain tumors maintains limited response to therapy and unfavorable clinical prognosis. The underlying molecular mechanisms of transcriptional re-programing that drive tumor heterogeneity are in the focus of research. This study reported an adaptive mechanism of malignant glioma in various metabolic environments, which potently drives transcriptional re-programming and tumor heterogeneity. In a first step, multiple biopsies (total n=138) were obtained from three *de-novo* glioblastoma multiforme patients and underwent a metabolic and transcriptional profiling. A metabolic landscape was explored marked by varying stages of hypoxia and creatine enrichment with a strong correlation to specific local transcriptional pattern. In the presence of environmental enriched creatine and GABA, the proneural expression subtype was mainly observed where lactate was found to be highly associated with the mesenchymal phenotype. In a second step, patient derived glioblastoma stem-like cells (GSC) were obtained and cultured in either hypoxia, creatine- supplemented or combined conditions for 24h and 48h. This environmental cell-model showed a synergistic effect of up-regulating the GABA signaling and a simultaneous inhibition of HIF signaling in a creatine-supplemented environment. This affected the transcriptional landscape with a shift toward a proneural subtype expression followed by a slowing of cell migration and proliferation. These findings were verified by additional HIF and GABA inhibition, which partially rescued the proneural shift. In a heterogeneous tumor, different stages of hypoxia and environmental creatine enrichment were observed, which required a permanent adaptation of the tumor to varying environmental conditions. These findings suggest that metabolic adaptation plays a major role in transcriptional heterogeneity of malignant brain tumors. Exploiting or targeting metabolic adaption could potentially serve as a future therapeutic option for malignant brain tumors.

METB-14. DOWN-REGULATION OF ACETATE METABOLISM TOWARDS FATTY ACIDS IN IDH1 MUTANT GLIOMA

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Acetate has recently been identified as a major alternative source of nutrients for glioblastoma and brain metastases. After cellular uptake, acetate is converted to acetyl-CoA, a key metabolic intermediate that fuels the TCA cycle and is an essential building block for the biosynthesis of fatty acids. Interestingly, the potential role of acetate in lower-grade glioma harboring the isocitrate dehydrogenase 1 mutation has not yet been elucidated. The goal of this study was therefore to investigate the role of acetate in fatty acid biosynthesis using a well-characterized genetically-engineered cell model that overexpresses either wild-type IDH1 (IDHwt) or mutant IDH1 (IDHmut): an immortalized normal human astrocyte (NHA)-based model. We used 1H and 13C magnetic resonance spectroscopy to quantify the flux of [1,2-13C]-acetate to 13C fatty acids. Our results indicated that the total levels of fatty acids were not significantly different between IDHmut and IDHwt NHA cells. However, the flux of 13C-labeled acetate towards fatty acids was significantly reduced by ~60% in IDHmut NHA cells relative to IDHwt NHA cells. To investigate this disconnect and understand the underlying biological mechanisms, we performed cell biological assays. Surprisingly, this decrease in acetate metabolism was associated with a drop in fatty acid synthase and ATP citrate lyase expressions, two enzymes involved in fatty acid synthesis from acetyl-CoA, in IDHmut NHA cells, whereas expression of acetyl-CoA synthase (AceS1), the cytosolic enzyme converting acetate to acetyl-CoA, was not altered. A significant drop in lipid droplet accumulation was also observed in IDHmut NHA cells as indicated using a spectrophotometric assay. Taken together, this points to alternate sources for fatty acids (e.g. glucose, glutamine, uptake from serum) in IDH1mut cells and suggests that fatty acids are preferentially directed towards cell membrane assembly. It also highlights the unique metabolic reprogramming of mutant IDH1 cells.

METB-15. REGULATION OF BIOENERGETICS THROUGH DUAL INHIBITION OF ALDEHYDE DEHYDROGENASE AND MITOCHONDRIAL COMPLEX I SUPPRESSES GLIOBLASTOMA TUMORSPHERES

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Glioblastoma (GBM), the most common primary brain tumor, continues to be associated with poor prognosis despite the best treatment modalities currently available. Targeted approaches for treating GBM attempted to date have consistently failed, highlighting the imperative for treatment strategies that operate on different mechanistic principles. Bioenergetics deprivation has emerged as an effective therapeutic approach for various tumors. We have previously found that cancer cells preferentially utilize cytosolic NADH supplied by aldehyde dehydrogenase (ALDH) for ATP production through oxidative phosphorylation (OxPhos). This study is aimed to examine therapeutic responses and underlying mechanisms of dual inhibition of ALDH and OxPhos against GBM. For inhibition of ALDH and OxPhos, the corresponding inhibitors, gossypol and phenformin were used. Biological functions, including ATP levels, stemness, invasiveness, and viability, were evaluated in GBM tumorspheres (TSs). Gene expression profiles were analyzed using microarray data. In vivo anticancer efficacy was examined in a mouse orthotopic xenograft model. Combined treatment of GBM TSs with gossypol and phenformin significantly reduced ATP levels, stemness, invasiveness, and cell viability. Consistently, this therapy substantially decreased expression of genes associated with stemness, mesenchymal transition, and invasion in GBM TSs. Supplementation of ATP using malate reversed these effects, whereas knockdown of ALDH1L1 mimicked them, suggesting that disruption of ALDH-mediated ATP production is a key mechanism of this therapeutic combination. In vivoefficacy confirmed remarkable therapeutic responses to combined treatment with gossypol and phenformin. In this study, we showed that combined treatment with gossypol and phenformin induces dual inhibition of bioenergetics by targeting ALDH and OxPhos, causing ATP depletion in GBM TSs. This regimen subsequently attenuated stemness, mesenchymal transition, and invasion, which are prominent features of GBM TSs, ultimately leading to a decrease in cell viability. Our findings suggest that dual inhibition of tumor bioenergetics is a novel and effective strategy for the treatment of GBM.

METB-16. ACQUISITION OF WARBURG PHENOTYPE IN IDH1-MUTATED GLIOMA AS A MECHANISM OF MALIGNANT TRANSFORMATION

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BACKGROUND: The Warburg effect is one of the hallmarks of cancer metabolism and its prevalence includes gliomas. The formation of lactate and upregulation of glycolysis has been the basis of many studies that either use ^{18}FDG PET or lactate as a readout of cancer progression. However, IDH1-mutated gliomas have been reported to have low glycolytic rates and minimal lactate production, due to the hypermethylation of the promoter region of LDHA. We postulated that initially, IDH1-mutated gliomas form very little lactate, but as they progress towards glioblastomas lactate production increases. **METHODS:** TS603 and BT142 cell lines harboring IDH1 mutation were cultured in DMEM/F12+ N2 supplement, glutamine, FGF and EGF, then either harvested for metabolomics and methylation analyses or (250,000 cells) were injected into 6-week-old SCID mice brains. MRI was used to monitor tumor size; when tumors reached 100 mm^3, mice were injected in the tail vain with 96 mM 1-^{13}C pyruvate which was hyperpolarized using Oxford Hypersense hyperpolarizer and chemical shift images were acquired immediately. Metabolite quantification was done using the Agilent LC/MS 6545 QTOF mass spectrometer. DNA methylation analysis was performed using Illumina Human Methylation 450 Bead Chip. **RESULTS AND CONCLUSIONS:** Our results demonstrate a unique example of IDH1-mutant glioma that transformed to a highly glycolytic, Warburg-like glioma that was associated with loss of global methylation and increased expression of glycolytic enzymes. Moreover, the glycolytic enzyme expression of the aggressive cell line correlates with the subset of patients that are IDH1 mutated and low-G-CIMP in TCGA database. The results of this study represent an example of malignant transformation that is linked with loss of methylation as a