(NAM) by NAMPT. D2HG in IDH-mutant tumors methylates and inactivates NAPRT, increasing dependence on NAMPT. Toxic side effects of NAMPT inhibition can be prevented by NA supplementation in healthy cells without NAPRT methylation. A1326133 is a recently described CNS-penetrant NAMPT inhibitor hypothesized to selectively eliminate IDH-mutant NAPRT-methylated gliomas, likely in combination with other therapies. Our group is looking for biomarkers of drug efficacy to augment individualized therapies. To that end, we sought to identify GBM cell lines with varying sensitivity to NAMPT inhibition. **METHODS:** Human non-immortalized astrocytes and human GBM cell lines were utilized from the Mayo Clinic Glioma patient-derived xenograft resource, including IDH-R132Hmutant lines (GBM164, 196) and IDH-WT lines (GBM6, 12, 76). Cell viability was analyzed after 4days incubation with the NAMPT inhibitor, A1326133 +/- Temozolomide (TMZ) or NA. IC50 for A1326133 was estimated based on intracellular ATP using Cell-Titer-Glo. **RESULTS:** Marked heterogeneity between lines was observed in response to A1326133 +/- NA or TMZ. Sensitive and resistant lines were identified among both IDH-mutant and IDH-WT cell lines. IC50s: GBM164, 12, 6, 196 and 76 were 5.6, 9.3, 39.2, 910, and 9455nM, respectively. NA partially rescued GBM164 by NA (IC50 increased to 20.8nM) but not GBM6 nor 12. IC50 for Human astrocytes was 221.7nM, but >10,000nM with NA. Addition of TMZ did not improve A1326133 efficacy. **CONCLUSION:** Our data illustrate the potential utility of NAMPT inhibition to kill a subset of IDH-WT and IDH mutant lines, but conflict with previously reported TMZ synergy and correlation with mutant IDH. NA may increase safety but could decrease efficacy in certain lines. Ongoing studies seek metabolic biomarkers of therapeutic efficacy to guide individualized therapy with NAMPT inhibitors.

DDRE-13. INHIBITION OF EXTRACELLULAR CARBONIC ANHYDRASES INHIBITS GLIOBLASTOMA CELL INVASION

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OBJECTIVE: Malignant gliomas metabolize glucose preferably by glycolysis which is in accordance with the Warburg effect. This induces a high demand of glucose combined with a significant lactic acid load. The hypoxia-inducible carbonic anhydrase (CA) IX has been shown to moderate the extrusion of hydrogen ions into the extracellular space. Since the acidification of the extracellular environment contributes to host tissue invasion due to activation of proteolytic enzymes, we hypothesized that CA IX plays an important role in malignant glioma Recently, specific small molecule inhibitors of this enzyme have been developed and may provide an innovative strategy for anti – invasive treatment. **METHODS:** Two established and 4 primary GBM cell lines (2 with mesenchymal and 2 with proneural transcriptional profile) were exposed to the CAIX inhibitor U104 under normoxic and hypoxic conditions. Cell toxicity was measured by ATP and crystal violet assay. For invasion assessment, a matrigel invasion chamber system with 8 µm pore size polycarbonate filter was used. CAIX expression was analyzed by quantitative RTPCR and Western Blot. **RESULTS:** Hypoxia significantly induced CAIX expression in all cell lines. Invasiveness increased significantly under hypoxic conditions in the mesenchymal cells ($p < 0.01$). Regardless of oxygenation status, the mesenchymal group displayed significantly higher invasiveness compared to the proneural group ($p = 0.006$). Looking at all cell lines, invasion is significantly inhibited by U104, both under normoxic and hypoxic conditions ($p < 0.01$). However, while the mesenchymal group showed the highest susceptibility to CAIX inhibition followed by the proneurally differentiated group, the established cell lines were entirely refractory to CAIX inhibition. **CONCLUSION:** Our data demonstrate that CAIX inhibition can effectively inhibit invasion in malignant glioma cells independent from oxygenation status, however the effects are significantly influenced by cell type specific biological features.

DDRE-14. DE-NOVO PURINE BIOSYNTHESIS IS A MAJOR DRIVER OF CHEMORESISTANCE IN GLIOBLASTOMA

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Glioblastoma is a primary brain cancer with a near 100% recurrence rate. Upon recurrence, the tumor is resistant to all conventional therapies, and because of this, 5-year survival is dismal. One of the major drivers of this high recurrence rate is glioblastoma cells' ability to adapt to complex changes within the tumor microenvironment. To elucidate this adaptation's molecular mechanisms, specifically during chemotherapy temozolomide, we employed chromatin immunoprecipitation followed by sequencing and gene expression analysis. We identified a molecular circuit in which the expression of ciliary protein ADP-ribosylation factor-like protein 13B (ALR13B) is epigenetically regulated to promote adaptation to chemotherapy. Immunoprecipitation combined with Liquid Chromatography-Mass Spectrometry binding partner analysis revealed that that ARL13B interacts with the purine biosynthetic enzyme inosine-5'-monophosphate dehydrogenase 2 (IMPDH2). Further, radioisotope tracing revealed that this interaction function as a negative regulator for purine salvaging. Inhibition of ARL13B-IMPDH2 interaction enhances temozolomide-induced DNA damage by forcing glioblastoma cells to rely on the purine salvage pathway. Targeting the ARLI3B-IMPDH2 circuit can be achieved using a Food and Drug Administration-approved drug, Mycophenolate Mofetil, that can block the IMPDH2 activity and enhance the therapeutic efficacy of TMZ. Our results suggest and support clinical evaluation of MMF in combination with TMZ treatment in glioma patients.

DDRE-15. THE EVOLUTIONARY ENIGMA OF FATTY ACID DESATURATION IN GLIOBLASTOMA

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Fatty acid desaturation is an enzymatic reaction in which a double bond is introduced into an acyl chain. Of the four functionally distinct desaturase subfamilies, the First Desaturase Family of enzymes introduce the first double bond into a saturated fatty acid, resulting in the synthesis of monounsaturated fatty acids (MUFA). MUFA are essential components of membrane and storage lipids and exert a profound influence on the fluidity of biological membranes. A disequilibrium in saturated to unsaturated fatty acid ratio alters cell growth, differentiation and response to external stimuli, and thus affects a range of pathologies including cancer. The most abundant and key First Desaturase Family enzyme is the delta 9 desaturate called Stearoyl Co-A Desaturase (SCD and SCD5 in humans, and SCD1-4 in mice). SCD desaturates Stearoyl-CoA (C18) and palmitoyl-CoA (C16) to oleoyl-CoA (C18:1) and palmitoyl-CoA (C16:1), respectively. Besides SCD, the only known First Desaturase in mammals with dual function is FADS2 which desaturates palmitate to Sapienate (C16:1, a positional isomer of palmitoleate) in skin cells. A recent study showed that some cancer cells can use FADS2 to bypass the SCD reaction. SCD and SCD5 are by far the most abundant desaturases expressed in the human brain. We made an unexpected discovery that SCD undergoes monoallelic codeletion with PTEN on chromosome 10, and is also highly methylated in glioblastoma (GBM). More surprisingly, all GBM cell lines with SCD codeletion/methylation (that expressed very little SCD protein) are completely resistant to SCD/SCD5 inhibition, yet their phospholipids contained abundant oleic acid. It is unknown if GBMs bypassed SCD, but retained the delta 9 desaturation reaction through a novel enzymatic activity. Our targeted and untargeted metabolomics studies revealed unexpected findings that cannot be explained by conventional wisdom, and may lead to identification of novel lipogenic targets in GBM.

DDRE-16. CYSTEINE IS AN ESSENTIAL AMINO ACID IN GLIOMAS

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BACKGROUND: Cysteine is a non-essential amino acid, since it can be synthetized from methionine through the transsulfuration pathway; moreover, cysteine is also uptake from the diet as cystine. We have investigated the metabolism of cysteine in glioma cell lines, and how cysteine/cystine-deprivation alters their antioxidant response in addition to the effect of this nutrient restriction to viability and proliferation *in vitro* and *in vivo*. **METHODS:** Cysteine metabolism was investigated through LCMS-based ^{13}C-tracing experiments involving different probes such as ^{13}C-*methyl*-Methionine, ^{13}C-C3-Cysteine, ^{13}C-C3,3'-Cystine, ^{13}C-C3-Serine and ^{13}C-U-Glutamine and the expression levels of key enzymes in the transsulfuration pathway were also explored. Finally, a mouse model of IDH1 mutant glioma was subjected to a cysteine/cystine-free diet and tumor metabolism was analyzed by LCMS. **RESULTS:** We demonstrated that exogenous cysteine/cystine are crucial for glutathione synthesis, and impact growth and viability. We also found that methionine cycle is disconnected from the transsulfuration pathway based on ^{13}C-tracing data and protein expression levels of cystathionine synthase and cystathioninase. Accordingly, cysteine-related metabolites such as GSH, involved in REDOX hemostasis, are downregulated, revealing a hypersensitive phenotype to ROS. Animal models upon a cysteine/cystine-free diet experienced an increase in survival and elevated levels of oxidative stress in tumor tissue. **CONCLUSION:** This results presented herein reveal an alternative therapeutic approach combining cysteine/cysteine-deprivation diets and treatments involving ROS production by limiting the ability of glioma cells to quench oxidative stress through dietary interventions.