(HER2+) breast cancer, and the unique brain microenvironment contributes to this therapy resistance. Nutrient availability can vary across tissues, therefore metabolic adaptations required for breast cancer growth in the brain microenvironment may also introduce liabilities that can be exploited for therapy. Here, we assessed how metabolism differs between breast tumors growing in the brain versus extracranial sites and found that fatty acid synthesis is elevated in breast tumors growing in the brain. We determine that this phenotype is an adaptation to decreased lipid availability in the brain relative to other tissues, which results in a site-specific dependency on fatty acid synthesis for breast tumors growing at this site. Genetic or pharmacological inhibition of fatty acid synthase (FASN) reduces HER2+ breast tumor growth in the brain, demonstrating that differences in nutrient availability across metastatic sites can result in targetable metabolic dependencies.

DDRE-08. NRF2/GLUTATHIONE METABOLISM AS A NOVEL THERAPEUTIC TARGET FOR IDH1-MUTATED GLIOMA

Yang Liu, Di Yu, Orieta Celiku, Aiguo Li, Mioara Larion, Chunzhang Yang; NCI, Bethesda, MD USA

BACKGROUND: *IDH1*-mutated glioma is a recently defined disease entity with distinctive patterns of tumor cell biology, metabolism, and resistance to therapy. Although *IDH1* mutations are highly prevalent in patients with WHO II/III glioma, curative molecular targeting approaches remain unavailable for this disease cluster. **METHODS:** In the present study, we investigated the glutathione *de novo* synthesis pathway through the TCGA patient cohort and patient-derived cell lines with *IDH1* mutation. The biologic function of nuclear factor erythroid 2-related factor 2 (NRF2) was analyzed by biochemistry and cell biology assays. Finally, NRF2 inhibitors were evaluated in *IDH1*-mutated cell lines and preclinical models as an experimental therapy. **RESULTS:** *IDH1* mutant neomorphic activity depletes the cellular pools of enzyme cofactors such as nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). The limitation of NAD(P) not only affects the anabolic reactions, but also results in oxidative stress and damages on DNA and protein. Further, we showed that the reprogrammed redox landscape results in constitutive activation of NRF2-governed cytoprotective pathways through the decoupling of NRF2 from its E3 ligase Kelch-like ECH-associated protein 1. NRF2 mediated the transcriptional activation of *GCLC*, *GCLM*, and *SLC7A11*, which not only strengthens the glutathione *de novo* synthesis, but also relieves the metabolic burden in *IDH1*-mutated cells. The importance of the glutathione synthesis is further confirmed through COX regression analysis on lower-grade glioma. Blockade of the NRF2/glutathione metabolic pathway synergizes with the elevated intrinsic oxidative stress, which results in overwhelming oxidative damage, as well as a substantial reduction in tumor cell proliferation and xenograft expansion. **CONCLUSION:** We report that the NRF2-guided cytoprotective pathways play pivotal roles in the disease progression of *IDH1*-mutated glioma. Targeting NRF2 and glutathione metabolism could be novel targeting strategies for *IDH1*-mutated glioma.

DDRE-09. THERAPEUTIC TARGETING OF PURINE METABOLISM IN DIPG

Ian Mersich[1,2], Biplab Dasgupta[2]; [1]University of Cincinnati Cancer and Cell Biology Graduate Program, Cincinnati, OH, USA, [2]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Diffuse intrinsic pontine glioma (DIPG) is an incurable brainstem malignancy in children with median survival less than 1 year and 5-year overall survival only 2 percent. Little progress has been made in treating this deadly disease due to its inoperable location and treatments aimed at targets defined in adult gliomas. Despite recent advances in genetic characterization of DIPGs there are still no targeted therapies that significantly improve overall survival. We recently generated a metabolic profile for patient-derived DIPG cell lines by integrating an untargeted metabolomics analysis with RNA-sequencing data from the same lines which demonstrated dysregulated purine metabolism in these cells. Furthermore, we have identified putative driver mutations common to DIPG patients as the direct cause for this metabolic alteration. Purine metabolism provides the basic components of nucleotides needed for tumor proliferation and thus considered a high-priority target in cancer treatment. De novo purine biosynthesis (DNPS) is a sequential ten step enzymatic process resulting in the production of inosine monophosphate. The DNPS enzymes co-localize into a metabolon known as the purinosome and our preliminary data demonstrates DIPG cell lines are selectively sensitive to pharmacological and genetic disruption of purinosome formation. Interestingly, antifolate compounds that inhibit DNPS, but do not disrupt purinosome assembly, are cytotoxic to both DIPG cells and normal cell types. Strikingly, cell viability could be rescued by purine supplementation when inhibiting this pathway with antifolates, however inhibition of DNPS by disruption of purinosome assembly could not be rescued. Metabolomics analysis showed DIPGs have a preference for generating GMP over AMP which is exacerbated when purinosome assembly is disrupted. This is likely due to the duel-role of the DNPS enzyme ADSL which is required for AMP production.

DDRE-10. METABOLIC TARGETING OF HUMAN GLIOBLASTOMA USING 5-AMINOLEVULINIC ACID (ALA)-MEDIATED SONODYNAMIC THERAPY: A FIRST-IN-HUMAN STUDY.

Stuart Marcus[1], Nader Sanai[2]; [1]SonALAsense, Inc, Berkeley, CA, USA, [2]Ivy Brain Tumor Center at the Barrow Neurological Institute, Phoenix, AZ, USA

Heme biosynthesis is altered in glioblastoma (GBM). Systemic dosing with ALA, the first committed molecule in the heme pathway, results in accumulation of the fluorescent intermediate, protoporphyrin IX (PpIX) only within tumor tissue (Gleolan label, 2019). PpIX is a photosensitizer that is effective in photodynamic therapy (PDT); in recurrent GBM patients, the safety and feasibility of ALA PDT has been demonstrated (Johansson A, et al. Lasers Surg Med 2013;45:225), although the practicality of this strategy in clinical care remains uncertain. Importantly, preclinical models of GBM show that PpIX is also a sonosensitizer and, in combination with transcranial MRI-guided focused ultrasound (MRgFUS), leads to non-ablative cytotoxic effects *in vivo* (Jeong EJ et al, Ultrasound in Medicine and Biology 2013:38;2143, Suehiro S et al, J Neurosurg 2018: 1377, Wu et al Nature Sci Reports 2019: 9;10465). The Ivy Brain Tumor Center is conducting a first-in-human study of 5-ALA sonodynamic therapy (SDT) for recurrent GBM (NCT 04559685). In this Phase 0/1 clinical trial, nontherapeutic, single-treatment SDT is administered prior to planned tumor resection. A Dose-Escalation Arm varies the power/energy of the MRgFUS while using a fixed time-interval from exposure to surgery. A subsequent Time-Escalation Arm varies the interval between MRgFUS and surgical resection, but fixes the power/energy of the delivered ultrasound. In both Arms, patient tumor tissue is assessed for sonodynamic and pharmacodynamic effects. In each patient, half of the tumor volume is not targeted with SDT and serves as an internal control. This first-in-human study will demonstrate the safety and feasibility of ALA sonodynamic therapy in GBM and may provide the first-ever biological evidence of sonosensitization in a brain tumor patient. If successful, this Phase 0 trial will introduce a new, metabolically-driven, GBM treatment modality that may be applicable to any brain tumor that selectively accumulates PpIX after ALA administration.

DDRE-11. TARGETING FATTY ACID BIOSYNTHESIS IN GLIOBLASTOMA

Katharina M. Eyme[1], Rudolph Neustadt[1], Christian E. Badr[1,2]; [1]Department of Neurology, Massachusetts General Hospital, Boston, MA, USA, [2]Neuroscience Program, Harvard Medical School, Boston, MA, USA

We recently provided evidence that endoplasmic reticulum (ER) stress promotes fatty acid (FA) biosynthesis in glioblastoma (GBM) cancer stem cells (GSCs). We determined that Stearoyl CoA Desaturase 1 (SCD), a key FA desaturase, is essential for regulating ER homeostasis in GSCs, and showed that these cells are highly susceptible to pharmacological perturbation of SCD activity. An impaired SCD activity leads to the toxic accumulation of saturated FA and activates cell death signaling mediated by the ER sensor Inositol-requiring enzyme 1 (IRE1). This in turn promotes an IRE1-mediated mRNA decay of key DNA damage repair genes and impairs the ability of GSCs to repair DNA damage caused by radiation or chemotherapy. Consequently, combining SCD inhibition with temozolomide (TMZ) leads to major cytotoxicity both in TMZ-sensitive, and TMZ-resistant patient-derived GBM cells. Pharmacological inhibition of SCD delivered through the nasal route in mice, had a remarkable therapeutic benefit in patient-derived orthotopic GSCs mouse models, yet the modest brain permeability of the currently available SCD inhibitors precludes their clinical translation. To overcome this challenge, we have recently acquired a first-in-class, clinically relevant SCD inhibitor. This compound has undergone extensive pharmacokinetic and pharmacodynamic studies which confirmed brain permeability, efficacy, and safety in small animals and non-human primates. We show that the combination of this SCD inhibitor with TMZ is effective both in cultured GSCs, and in preclinical GSCs orthotopic mouse models. Our results support the clinical investigation of this new class of SCD inhibitors, in combination with TMZ, in patients diagnosed with GBM.

DDRE-12. HETEROGENOUS RESPONSE OF IDH-MUTANT AND IDH-WT GLIOMA TO NAMPT INHIBITION

Masum Rahman, Renee Hirte, Ian Olson, Moustafa Mansour, Samar Ikram, Amanda MunozCasabella, Rujapope Sutiwisesak, Lucas Carlstrom, Arthur Warrington, Karishma Rajani, Jann Sarkaria, Terry Burns; Mayo Clinic, Rochester, MN, USA

BACKGROUND: NAD+ is required for cell metabolism and DNA repair. It is generated from nicotinic acid (NA) by NAPRT and from Nicotinamide

(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

Martin Proescholdt[1], Qiu Zhenwei[1], Lohmeier Annette[1], Schmidt Nils-Ole[1], Merrill Marsha[2]; [1]University Hospital Regensburg, Regensburg, By, Germany, [2]Surgical Neurology Branch, NIH, Bethesda, MD, USA

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

Atique Ahmed; Northwestern University, Chicago, IL, USA

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

Nicole Oatman, Biplab Dasgupta; Cincinnati Children's, Cincinnati, OH, USA

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

Victor Ruiz-Rodado[1], Tyrone Dowdy[1], Jinkyu Yung[1], Ana Dios-Esponera[2], Adrian Lita[1], Tamalee Kramp[1], Kevin Camphausen[1], Mark Gilbert[1], Mioara Larion[1]; [1]National Cancer Institute, Bethesda, MD, USA, [2]Fred Hutchinson Cancer Research Center, Seattle, WA, USA

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.