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Mutations in *IDH1* and *IDH2* genes are common in low grade gliomas and secondary GBM and are known to cause a distinct epigenetic landscape in these tumors. To interrogate the epigenetic vulnerabilities of *IDH*-mutant gliomas, we performed a chemical screen with inhibitors of chromatin modifiers and identified 5-azacytidine, Chaetocin, GSK-J4 and Belinostat as potent agents against primary *IDH1*-mutant cell lines. Testing the combinatorial efficacy of these agents, we demonstrated GSK-J4 and Belinostat combination as a very effective treatment for the *IDH1*-mutant glioma cells. Engineering established cell lines to ectopically express *IDH1R132H*, we showed that I*DH1R132H* cells adopted a different transcriptome with changes in stress-related pathways that were reversible with the mutant IDH1 inhibitor, GSK864. The combination of GSK-J4 and Belinostat was highly effective on *IDH1R132H* cells, but not on wt glioma cells or nonmalignant fibroblasts and astrocytes. The cell death induced by GSK-J4 and Belinostat combination involved the induction of cell cycle arrest and apoptosis. RNA sequencing analyses revealed activation of inflammatory and unfolded protein response pathways in *IDH1*-mutant cells upon treatment with GSK-J4 and Belinostat conferring increased stress to glioma cells. Specifically, GSK-J4 induced ATF4-mediated integrated stress response and Belinostat induced cell cycle arrest in primary *IDH1*-mutant glioma cells; which were accompanied by *DDIT3*/CHOP-dependent upregulation of apoptosis. Moreover, to dissect out the responsible target histone demethylase, we undertook genetic approach and demonstrated that CRISPR/Cas9 mediated ablation of both *KDM6A* and *KDM6B* genes phenocopied the effects of GSK-J4 in *IDH1*-mutant cells. Finally, GSK-J4 and Belinostat combination significantly decreased tumor growth and increased survival in an orthotopic model in mice. Together, these results suggest a potential combination epigenetic therapy against *IDH1*-mutant gliomas.

DDRE-04. THE COMBINED TREATMENT OF L-ASPARAGINASE AND 6-DIAZO-5-OXO-L-NORLEUCINE INHIBIT THE PROLIFERATION OF TEMOZOLOMIDE-SENSITIVE OR RESISTANT GLIOBLASTOMA CELLS

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Glioblastoma is one of the aggressive brain tumors with a 5-year survival rate of < 10%. The standard treatment is maximal safe resection, followed by radiation therapy and temozolomide (TMZ). Clinically, the resistance to TMZ is a big problem. Cancer cells have been revealed to show different metabolism from normal cells. The object of this study is to evaluate whether cancer metabolism, especially asparagine, could be a new target of treatment in primary and recurrent glioblastoma. Glioblastoma cells (U251 and U87) were treated with L-asparaginase and/or 6-diazo-5-oxo-L-norleucine (DON). L-asparaginase converts asparagine into aspartate and depletes asparagine. DON is a glutamine analog that inhibits several glutamine-utilizing enzymes, including asparagine synthetase. L-asparaginase or DON suppressed the proliferation of U251, and U87 cells in a dose-dependent manner. Combined treatment with these drugs had a synergistic antiproliferative effect in these cell lines. The effect was counteracted by exogenous asparagine. The combined treatment induced greater apoptosis and autophagy than did single-drug treatment. Several clones of TMZ-resistant U251 were obtained after long treatment of TMZ to U251. The expression of MSH6, one of the mismatch repair proteins, was suppressed in these resistant clones. The synergistic effect of L-asparaginase and DON was detected in these U251-derived TMZ-resistant clones. These results suggest that the combination of L-asparaginase and DON could be a new therapeutic option for patients with primary and recurrent glioblastoma.

DDRE-05. STEAROYL COA DESATURASE IS ESSENTIAL FOR REGULATION OF ENDOPLASMIC RETICULUM HOMEOSTASIS AND TUMOR GROWTH IN GLIOBLASTOMA CANCER STEM CELLS

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INTRODUCTION: Emerging evidence suggest that, in addition to glucose, fatty acids can also drive glioma growth. Increased lipid synthesis is one of the metabolic hallmarks of cancer, and indeed, unsaturated fatty acids (UFA) are particularly abundant in glioblastoma. However, the exact role of fatty acids in GBM tumors remains unclear. Blocking fatty acids synthesis can present a new therapy for GBM. **METHODS:** Through targeted inhibitors screening on glioma stem cells (GSCs), we found that they are highly susceptible to Stearoyl CoA Desaturase 1 (SCD1) inhibitors. SCD1 is a key enzyme responsible for the conversion of saturated fatty acids (SFA) to UFA. 1) Through cell-based assays and immunoblot analyses, we tried to understand the role of UFA, SFA and SCD1 in GSCs differentiation and proliferation. We investigated the mechanism between fatty acids and tumor growth through ER stress modulation linked with SCD1 expression. 2) As we found that GSCs are highly susceptible to SCD1 inhibition, we tested CAY, SCD1 inhibitor, in GSCs orthotopic mouse models and assess its effect on tumor growth and overall survival. **RESULTS:** We found that GSCs with extensive self-renewal capacity have an increased dependence on SCD1 activity. Through immunoblot analyses, we demonstrated that SCD1 inhibition exacerbates ER stress through accumulation of SFA and SCD1-mediated UFA synthesis mitigates ER stress. Survival analyses between SCD1 inhibitor-treated group and control group showed significant survival benefit in SCD1-inhibitor-treated group, in both mesenchymal (p=0.008, 35 days vs 18) and proneural (p=0.0002) type glioma cells (n=8/groups). **CONCLUSIONS:** We demonstrate that SCD1, the fatty acid desaturase, is essential for the maintenance of glioblastoma cancer stem cells. SCD1 is activated by ER stress and exerts a cytoprotective function by regulating ER homeostasis, thus favoring survival and tumor growth. Pharmacological targeting of SCD1 exhibits potent therapeutic efficacy in brain tumor mouse models.

DDRE-06. REGULATION OF TUMOR MICROENVIRONMENT VIA ENDOTHELIAL-TO-MESENCHYMAL TRANSITION BLOCKADE IN GLIOBLASTOMA-ASSOCIATED BRAIN ENDOTHELIAL CELLS

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Glioblastoma multiforme (GBM) is a malignant brain tumor noted for its extensive vascularity, aggressiveness, and highly invasive nature. Glioma stem cells (GSC) are a subpopulation of cells resistant to treatments and considered responsible for tumor recurrence. GSC are found in the vascular niches of the tumors, where endothelial cells (EC) secrete factors that stimulate GSC self-renewal. There are several studies regarding the effects of the vasculature on CSC and tumorigenesis, but little is known about how GSC affects the vasculature. Resistance to therapies and tumor recurrence greatly rely on the pro-angiogenic nature and aberrant vasculature of GBM. The endothelial-to-mesenchymal transition (EndMT) supports the pro-angiogenic and invasive characteristics of GBM. Hence, blocking the EndMT would be a promising approach to inhibit tumor progression and recurrence. We have examined the dynamic cross-talk between GSC and EC during EndMT. We demonstrate that GSC induce EndMT in brain endothelial cells (BEC), through a collaboration between TGF-β and Notch pathways, nicotinamide N-methyltransferase upregulation and other key signaling routes. Elucidating the cells and molecular pathways responsible for this process represents a milestone in the understanding of the tumor microenvironment and will help develop novel treatments in glioma therapy. One promising treatment, developed by our research group, is the conjugate of temozolomide and perillyl alcohol (POH), NEO212. This drug blocks EndMT induction in BEC and reverts the mesenchymal phenotype of tumor-associated BEC (TuBEC), reducing the invasiveness and pro-angiogenic properties of GBM in vitro and in vivo. We are currently performing Investigational New Drug (IND)-enabling studies, and we foresee that NEO212 will be of great clinical value for the treatment of GBM.

DDRE-07. FATTY ACID SYNTHESIS IS REQUIRED FOR BREAST CANCER BRAIN METASTASIS

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Brain metastases are refractory to therapies that otherwise control systemic disease in patients with human epidermal growth factor receptor 2

(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

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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

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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.

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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

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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

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