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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