quiring high resolution metabolic maps similar to anatomical MRI is challenging due to low metabolite concentrations, and alternative approaches that increase resolution by post-acquisition image processing can mitigate this limitation. We developed deep learning super-resolution MRI spectroscopic imaging (MRSI) to map tumor metabolism in patients with mutant IDH glioma. We used a generative adversarial network (GAN) architecture comprised of a Unet neural network as the generator network and a discriminator network for adversarial training. We trained a large data set of 9600 images with realistic quality for acquired MRSI to effectively train the deep learning model to upsample by a factor of four. Two types of training were performed: 1) using only the MRSI data, and 2) using MRSI and prior information from anatomical MRI to further enhance structural details. The performance of super-resolution methods was evaluated by peak SNR (PSNR), structure similarity index (SSIM), and feature similarity index (FSIM). After training on simulations, GAN was evaluated on measured MRSI metabolic maps acquired with resolution 5.2x5.2 mm$^2$ and upsampled to 1.3x1.3 mm$^2$. The GAN trained only on MRSI achieved PSNR = 27.94, SSIM = 0.88, FSIM = 0.89. Using prior anatomical MRI improved GAN performance to PSNR = 30.75, SSIM = 0.90, FSIM = 0.92. In the patient measured data, GAN super-resolution metabolic images provided clearer tumor margins and made apparent the tumor metabolic heterogeneity. Compared to conventional image interpolation such as bicubic or total variation, deep learning methods provided sharper edges and less blurring of structural details. Our results indicate that the proposed deep learning method is effective in enhancing the spatial resolution of metabolite maps which may better guide treatment in mutant IDH glioma patients.

BIMG-23. SINGLE-VOXEL VERSUS MULTI-SLICE MRSI IN PATIENTS WITH GLIOMA ON A KETOGENIC DIET INTERVENTION
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BACKGROUND: Ketogenic diet therapies (KDTs) may be beneficial by exploiting glioma metabolic vulnerabilities. The GLioma modified Atkins-based Diet study (GLAD; NCT02286167) evaluated systemic and cerebral (MR spectroscopy) biomarkers to determine the feasibility and biological effects of a KDT in glioma patients. While we observed metabolic changes in tumor and normal brain after KDT using single-voxel MRS (SV-MRS), optimal voxel placement was not always achieved. AIMS: We performed an exploratory analysis comparing cerebral metabolic changes using multi-slice MRSI (MS-MRS) versus SV-MRS acquisition.

METHODS: We evaluated four patients from the GLAD study (mean age 39 years; 2 female, 3 AA IDH-mutant, 1 GBM IDH-wildtype) who underwent MRS at baseline and following eight weeks of KDT. SV-MRS (sLASER, TR/TE 2.0x34ms) was acquired from a 2x2x2cm voxel placed in the residual tumor and the contralateral homologous brain. MS-MRSI was acquired with a multi-slice spin echo sequence (TR/TE 3.6/144ms, 4 slices, nominal resolution 13x7x7mm, SENSE factor 3) and maps of total choline (tCho), total N-acetyl-aspartate (tNAA), and lactate (Lac) were reconstructed and normalized relative to creatine. Metabolite levels total choline (tCho), total N-acetyl-aspartate (tNAA), and lactate (Lac) were measured on the MS-MRSI maps using a region of interest placed in the same areas studied with the SV-MRS. RESULTS: Lactal tCho and tNAA levels showed strong correlation between SV-MRS and MS-MRSI both at baseline (Pearson’s r=0.92 and 0.97, respectively) and after 8 weeks of KDT (r=0.96 and 0.84, respectively). tCho and tNAA correlated less robustly between SV-MRS and MS-MRSI in the contralateral region (r=0.56–0.96). Lactal Lc was significantly lower after KDT (1.01±0.48 before vs 0.54±0.24, paired t-test p=0.02). CONCLUSIONS: While SV and MS-MRSI provided generally concordant lesion results, MS-MRSI offers added potential to map regional variations not captured by SV-MRS and thus may better define the control regions. MS-MRSI detected a decrease in tumoral lactate levels following study intervention, suggesting KDT-related changes in tumoral energy metabolism.

METABOLIC DRUG TARGETS, RESISTANCE

DDRE-02. SMOOTHED-ACTIVATING LIPIDS DRIVE RESISTANCE TO CDK4/6 INHIBITION IN HEDGEHOG-ASSOCIATED MEDULLOBLASTOMA
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BACKGROUND: Medulloblastoma is an aggressive pediatric brain tumor that is associated with misactivation of the Hedgehog (HH) pathway. Our lab has shown that CDK6, a critical activator of the cell cycle, is a direct transcriptional target of oncogenic HH signaling, and that inhibiting CDK6 blocks the growth of HH-associated medulloblastoma in mice. A clinical trial exploring the efficacy of CDK6 inhibition in medulloblastoma patients is underway, but prior attempts to target the HH pathway in medulloblastoma have been encumbered by resistance to molecular monotherapy. Thus, we sought to identify mechanisms of resistance to CDK6 inhibition in HH-associated medulloblastoma.

METHODS: We performed orthogonal CRISPR and CRISPR interference screens in HH-associated medulloblastoma cell lines and primary isogenic inhibitors of CDK6. Metabolic sequencing of HH-associated medulloblastomas with genetic deletion of CDK6 in vivo. Mechanistic and functional validation of resistance pathways was performed using CRISPR interference, immunoblotting, immunofluorescence, genetics, and pharmacology. Lipid quantification was carried out by ultra-high performance liquid chromatography-tandem mass spectrometry.

RESULTS: Our results reveal that decreased ribosomal protein expression underlies resistance to CDK6 inhibition in HH-associated medulloblastoma, leading to endoplasmic reticular (ER) stress and activation of the unfolded protein response (UPR). We show that ER stress and the UPR increase the activity of enzymes producing Smoothened-activating sterol lipids that sustain oncogenic HH signaling in medulloblastoma despite CDK6 inhibition. These discoveries suggest that combination molecular therapy against CDK6 and HSD11B2, an enzyme producing Smoothened-activating lipids, may be an effective treatment for HH-associated medulloblastoma. In support of this hypothesis, we demonstrate that concurrent genetic deletion or pharmacological inhibition of CDK6 and HSD11B2 additively blocks the growth of multiple models of HH-associated medulloblastoma in mice.

CONCLUSIONS: Smoothened-activating lipid biosynthesis underlies resistance to CDK6 inhibition in HH-associated medulloblastoma, revealing a novel combination therapy to treat the most common malignant brain tumor in children.

DDRE-03. IDH1-MUTANT GBM CELLS ARE HIGHLY SENSITIVE TO COMBINATION OF KDM6A/B AND HDAC INHIBITORS
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BACKGROUND: Mutant IDH1 (IDH1$^{132R}$) gliomas have characteristic genetic and metabolic profiles and exhibit phenotype that is distinct from their wild-type counterparts. The glutamine/glutamate pathway has been hypothesized as a selective therapeutic target in IDH1$^{132R}$ gliomas. However, little information exists on the contribution of this pathway to the formation of D-2HG and its role in the metabolic consequences of inhibiting this pathway. METHODS: We employed an untargeted metabolic profiling approach in order to detect metabolic changes arising from glutaminase (GLS) inhibition treatment. We performed combined Nuclear Magnetic Resonance and Liquid Chromatography-Mass Spectrometry approach, explored the fate of glutamine and glucose under treatment with CB839 a glutaminase-GLS-inhibitor and their respective contributions to D-2HG formation. RESULTS AND CONCLUSIONS: The effects of CB839 on cellular proliferation differed among the cell lines tested, leading to designations of GLS-inhibition super-sensitive, -sensitive or -resistant. Our data indicates a decrease in the production of downstream metabolites of glutamate, including those involved in the TCA cycle, when treating the sensitive cells with CB839 (glutaminase-GLS-inhibitor). Notably, CB839-sensitive IDH1$^{132R}$ cells respond to GLS inhibition by upregulating glycolysis and lactate production. In contrast, CB839-resistant IDH1$^{132R}$ cell lines do not rely only on glutamine for the sustenance of TCA cycle. In these cells, glucose contribution to TCA is enough to compensate the downregulation of glutamine-derived TCA metabolites. This investigation reveals that the glutamine/glutamate pathway contributes differentially to D-2HG in a cell-line dependent fashion on a panel of IDH1$^{132R}$ cell lines. Further, these results demonstrate that there is a heterogeneous landscape of IDH1$^{132R}$ metabolic phenotypes. This underscores the importance of detailed metabolic profiling of IDH1$^{132R}$ patients prior to the decision to target glutamine/glutamate pathway clinically.
Abstracts

DDRE-04. THE COMBINED TREATMENT OF L-ASPARAGINASE AND BENZODI-5-OKX-L-NORLEUCINE INHIBITS THE PROLIFERATION OF TEMOZOLOMIDE-SENSITIVE OR RESISTANT GLOBLASTOMA 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, and 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 5-diazoo-L-norleucine (DON). L-asparaginase (DON) and asparaginase 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-resistant 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 GLOBLASTOMA 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 often observed in malignant glioma cells, and saturated fatty acids (SFA) 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) inhibitor. 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 behind the drug induced 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 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 fostering 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-MESENCHYAL TRANSITION BLOCKADE IN GLOBLASTOMA-ASSOCIATED BRADN ENDOTHELIAL CELLS
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Glioblastoma multiforme (GBM) is a malignant brain tumor noted for its extensive vascularity, aggressiveness, and highly invasive nature. Gloma stem cells (GSC) are a population 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 CSC 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 GBC. Hence, blocking the EndMT would be a promising approach to inhibit tumor progression and recurrence. We have examined the dynamic crosstalk 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 key potential contributor to 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 penrilyl alcohol (POH), NEO212. This drug blocks and reverts the mesenchymal condition 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