Title
DDRE-31. MITOCHONDRIAL TRAFFICKING AS A TARGET FOR GBM THERAPY

Journal
Neuro-Oncology, 23(Supplement_6)

ISSN
1522-8517

Authors
Lepe, Javier
Douglas, Christopher
Lomeli, Naomi
et al.

Publication Date
2021-11-12

DOI
10.1093/neuonc/noab196.315

Peer reviewed

agent alone. Similarly, in a temozolomide-refractory T98G GBM subcutaneous xenograft model, ST101 (41.6% TGI) in combination with TMZ (< 5% TGI) resulted in significant anti-GBM response (72.4% TGI). These data emphasize the potential of ST101 as a potent peptide therapeutic for GBM.

DDRE-31. MITOCHONDRIAL TRAFFICKING AS A TARGET FOR GBM THERAPY

Javier Lepe[1], Christopher Douglas[1], Naomi Lomeli[2], Kaijun Di[1], Bhaskar Das[3], and Daniela Bota[1]; [1]University of California Irvine, Irvine, CA, USA, [2]Department of Neurology, University of California, Irvine, Irvine, CA, USA, [3]Long Island University, Bronx, NY, USA

Glioblastoma (WHO Grade IV glioma) is the most aggressive brain cancer. The current standard of care treatment includes surgery, radiation, and chemotherapy. Tumor recurrence is almost inevitable as less than 50% of patients survive more than two years. The low survival rate poses a dire need to develop an effective therapy for GBM patients. GBM cells are resistant to treatment, as they activate their DNA damage response mechanisms to overcome the effects of radiation and temozolomide (TMZ) treatments. Recurrent tumors can arise from slow cycling and self-renewing stem/tumor-initiating cells resistant to radiation and TMZ. No second-line therapy was proven to prolong survival after TMZ failure. Magmas (Mitochondria-associated protein involved in granulocyte-macrophage colony-stimulating factor signal transduction) is a subunit of the TIM23 complex regulating precursor protein trafficking into the mitochondrial matrix. Magmas is encoded by pam16, known to be upregulated in human pituitary adenomas, prostate cancer and GBM. Previous studies have demonstrated that Magmas negatively regulates the stimulatory activity of Pam18, which in turn stimulates the ATPase activity of mitochondrial heat shock protein 70 (mtHsp70). No small molecules targeting Magmas are in clinical use. We developed a novel small molecule inhibitor (BT9) that has been specifically designed to inhibit Magmas binding to Pam18. BT9 induces apoptosis through cleavage of caspase-3, reduced mitochondrial respiration and glycolysis. Our recent findings also demonstrate that BT9 treatment reduced protein trafficking of Lon protease into the mitochondrial matrix. Pretreatment of glioma cells with BT9 sensitizes cells to radiation treatment and enhances the TMZ activity. BT9 can cross the blood-brain-barrier and improve survival in intracranial glioma PDX models. BT9 has potential therapeutic value by directly dysregulating mitochondrial function in GBM, enhancing radiation and chemotherapy response, and improving survival in a relevant animal model.

DDRE-32. SETD2 HISTONE METHYLTRANSFERASE MUTATION STATUS PREDICTS TREATMENT RESPONSE IN GLIOBLASTOMA: STRATEGIES TO OVERCOME CHEMORESISTANCE

Michael Goldstein, Nishanth Gabriel, Matthew Inkman, Jin Zhang, and Sonika Dahiya; Washington University in St. Louis, Saint Louis, MO, USA

A major challenge in GBM treatment is tumor resistance to radiation and chemotherapy. A hallmark of GBM is the frequent mutation of epigenetic modifiers resulting in alteration of epigenetic signaling pathways. However, the effect of epigenetic signaling on chemotherapy response in GBM remains unknown. SETD2 is a histone methyl transferase that facilitates H3K36 trimethylation. Here, we unveil the role of SETD2 mutations that frequently occur in GBM in tumor resistance to temozolomide chemotherapy. Targeted sequencing of the SETD2 gene in GBM tumor samples revealed that SETD2 mutations are associated with reduced overall survival in patients with methylated MGMT (methyl-guanine methyl transferase) promotor who received temozolomide. Consequently, we demonstrate that loss of SETD2 results in reduced H3K36me3 levels and a profound temozolomide resistance in GBM cells. MGMT-deficient tumors can acquire chemoresistance due to disrupted mismatch repair (MMR), a DNA repair pathway that converts primary temozolomide-induced DNA lesions into toxic DNA double-strand breaks. Strikingly, we found that SETD2 loss abrogates the expression of the MMR factor MSH6 indicating that chemoresistance in SETD2-deficient cells us due to disrupted MMR. Mechanistically, we show that SETD2 regulates MMR by promoting transcription of the *MSH6* gene in GBM. Epigenetic modifiers have specific antagonists capable of reversing chromatin alterations induced by these modifiers. This provides a unique opportunity to restore chemotherapy response in SETD2-mutant GBM by targeting the antagonists of SETD2. We demonstrate that combined targeting of H3K36me3-specific histone de-methylases KDM4A and NO66 restores H3K36me3 levels along with MSH6 expression and sensitivity to temozolomide in SETD2-deficient GBM cells. Thus, our findings establish SETD2 mutation as a novel molecular marker predictive of chemotherapy response in GBM and provide a framework for a novel approach to overcome chemotherapy resistance in this malignant brain tumor by targeting an epigenetic pathway.

DDRE-33. PRECLINICAL THERAPEUTIC EFFICACY OF THE NOVEL BLOOD BRAIN BARRIER PENETRANT ATR INHIBITOR LR02 IN GLIOBLASTOMA

Dimpy Koul[1], Veerakumar Balasubramaniyan[1], Xiaolong Li[1], Sabbir Khan[1], Davide Guggi[2], Thomas Mehrling[2], and John DeGroot[1]; [1]UT MD Anderson Cancer Center, Houston, TX, USA, [2]Laevoroc Oncology, Boesch, Switzerland

Glioblastoma (GBM) remains an incurable tumor with median overall survival of 15 months despite radiation and alkylating temozolomide (TMZ) chemotherapy. DNA damage response (DDR) pathways are among the most important key players of oncogenic mutations associated with resistance to both chemotherapy and radiation in GBM. The high frequency of alterations in DDR pathways in GBM suggests that its inhibition by DDR inhibitors may render GBM cells more susceptible to DNA damaging interventions. Here, we report the preclinical *in vitro* and *in vivo* activity of a novel, orally bioavailable Ataxia-telangiectasia mutated serine/threonine protein kinase and Rad3-related (ATR) inhibitor LR02 (Laevoroc Oncology) in a panel of 15 well-characterized glioma stem-like cells (GSCs). Effects on cell proliferation, survival and tumor formation were analyzed following treatment with LR02. Growth inhibition was time- and dose-dependent with a 3-day exposure resulting in a growth inhibitory IC50 (gIC50) in the low nM range in all the glioblastoma cell lines tested. LR02 inhibited growth of GSCs at IC_{50} values ranging from 500nmol/L to-~2umol/L. Additional studies showed that temozolomide sensitized GSC to LR02. Importantly, we demonstrate that MGMT promotor methylation status was associated with cellular response to LR02 treatment with preferential inhibition of cell growth in MGMT promotor methylated (MGMT deficient) cell lines. LR02 showed efficacy and survival benefit in a GSC262 (MGMT methylated) orthotopic model of GBM. Further administration of LR02 further enhanced the *in vivo* antitumor efficacy of temozolomide (TMZ) against GBM using the GSC262 model demonstrating that ATR inhibitor LR02 may enhance alkylating agent-mediated cytotoxicity and provide a novel treatment combination for GBM patients. Our present findings establish that the ATR inhibitor LR02 can specifically be used in tumors with MGMT deficiency when combined with alkylating chemotherapy. Further studies are ongoing to evaluate the potential of LR02 to overcome radiation and chemotherapy resistance in glioblastoma.

DDRE-34. RIBONUCLEOTIDE REDUCTASE REGULATORY SUBUNIT M2 AS A DRIVER OF GLIOBLASTOMA TMZ-RESISTANCE THROUGH MODULATION OF DNTP PRODUCTION

Ella Perrault[1], Jack Shireman[1], Peiyu Lin[1], Eunus Ali[1], Andrew Zolp[1], Shreya Budhiraja[1], Cheol Park[1], Shivani Baisiwala[1], David James[1], Issam Ben-Sahra[1], Sebastian Pott[2], Anindita Basu[2], and Atique Ahmed[3]; [1]Feinberg School of Medicine, Chicago, IL, USA, [2]University of Chicago, Chicago, USA, [3]Department of Neurological Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Glioblastoma (GBM) remains one of the most resistant and fatal forms of cancer. Previous studies examine pre- and post-tumor recurrence; however, it is incredibly difficult to study tumor evolution during therapy where resistance develops. To investigate this, our lab performed a single-cell RNA-sequencing screen before, during, and after temozolomide-based (TMZ) chemotherapy in a patient-derived xenograft (PDX) model *in vivo*. Our analysis found 149 genes uniquely expressed during TMZ-therapy compared to pre- and post-therapy ($p< 0.0001$). Of these, the ribonucleotide reductase (RNR) gene family stood out due to the preferential switch to Ribonucleotide Reductase Regulatory Subunit M2 (RRM2) during therapy. Classically, RRM2, or its isoform RRM2B, forms a complex with RRM1 to create an RNR, mediating deoxynucleoside triphosphate (dNTP) production. Our single-cell data revealed that GBM cells rely on RRM1-RRM2 interaction during therapy, but switch to RRM1-RRM2B in post-therapy recurrent GBM. *In vitro*, RRM2-knockdown cells increased TMZ susceptibility, whereas RRM1- and RRM2B-knockdowns were more resistant to TMZ ($p< 0.001$). Immunocytochemistry found elevated γH2AX fluorescence in RRM2-knockdowns after TMZ treatment, signifying reduced DNA repair capacity compared to the control ($p< 0.001$). To understand the mechanism of RRM2-mediated chemoresistance, targeted metabolomics was applied to quantify dNTP signatures during TMZ-therapy. In response to TMZ, dCTP and dGTP production in GBM cells increased 100-fold and 80-fold respectively ($p< 0.001$). RRM2-knockdowns produced significantly less dCTP and dGTP ($p< 0.0001$). By supplementing RRM2-knockdowns with dCTP and dGTP, TMZ-susceptibility was rescued, suggesting that RRM2 drives chemoresistance by promoting production of these two nucleotides. *In vivo*, following intracranial injection of GBM cells, mice treated with the RRM2 inhibitor Triapine with TMZ survived longer than those treated with TMZ alone, indicating promising clinical opportunities in targeting RRM2 ($p< 0.0001$). Overall, our data present a novel understanding of how RRM2 activity is altered during therapeutic stress to counteract TMZ-induced DNA damage.