Temozolomide resistance in glioblastoma

- Integrated Workflow for Drug Repurposing in Glioblastoma: Computational Prediction and Preclinical Validation of Therapeutic Candidates
- MiR 329/449 Suppresses Cell Proliferation, Migration and Synergistically Sensitizes GBM to TMZ by Inhibiting Src/FAK, NF-kB, and Cyclin D1 Activity
- Epigenetic Alterations in Glioblastoma Multiforme as Novel Therapeutic Targets: A Scoping Review
- An In Vivo Model of Recurrent Glioblastoma
- Exosomal circular RNAs as drivers of temozolomide resistance in glioblastoma: Mechanisms and implications
- Unlocking glioblastoma: breakthroughs in molecular mechanisms and next-generation therapies
- A novel approach to enhance glioblastoma multiforme treatment efficacy: non-coding RNA targeted therapy and adjuvant approaches
- Radio-chemotherapy and metformin selectively modulate the heterogeneous landscape of glioma with ribosome biogenesis, long non coding RNA and immune-escape markers as major player

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Temozolomide resistance in glioblastoma (GBM) represents a significant challenge in the treatment of this aggressive brain tumor.

Mechanisms of Resistance

1. **MGMT Promoter Methylation Status**:
    1. **MGMT promoter methylation**: This DNA repair enzyme removes methyl groups from the O6 position of guanine, counteracting the cytotoxic effects of TMZ. MGMT promoter methylation silences the gene, reducing its activity and increasing TMZ sensitivity.
    2. **Unmethylated Promoter**: High MGMT expression correlates with resistance to TMZ.

2. **Mismatch Repair (MMR) Deficiency**:
    1. Tumor cells with defective MMR fail to recognize and repair TMZ-induced DNA damage, leading to drug resistance.

3. **Base Excision Repair (BER) Pathway**:
    1. The BER pathway repairs TMZ-induced N7-methylguanine and N3-methyladenine lesions, contributing to resistance.

4. **DNA Damage Response (DDR) and Cell Cycle Arrest**:
    1. Dysregulation of DDR pathways, including checkpoint kinases and p53, impacts TMZ efficacy.
    2. Mutations in **TP53** can impair apoptotic responses to DNA damage.

5. **Epigenetic and Genetic Alterations**:

1. Aberrations in signaling pathways, such as **PI3K/AKT/mTOR** or **RAS/RAF/ERK**, can enhance survival and drug resistance.
2. Epigenetic changes like histone modifications may contribute to resistance mechanisms.

6. **Tumor Microenvironment**:

1. Hypoxia and immune suppression within the GBM microenvironment can promote resistance to TMZ.
2. Interaction with surrounding stromal cells and secretion of cytokines may also contribute.

7. **Cancer Stem Cells (CSCs)**:

1. Glioblastoma stem-like cells are inherently more resistant to TMZ due to enhanced DNA repair, efflux pumps, and stemness-associated pathways.

Strategies to Overcome Resistance

1. **MGMT Inhibition**:

1. Direct MGMT inhibitors (e.g., O6-benzyl guanine) have been investigated but are associated with systemic toxicity.

2. **Epigenetic Modulation**:

1. Histone deacetylase (HDAC) inhibitors or DNA methyltransferase inhibitors may re-sensitize tumors to TMZ.

3. **Targeting DNA Repair Pathways**:

1. Inhibitors of PARP, ATR, and other DNA repair enzymes are under clinical investigation.

4. **Combination Therapies**:

1. Combining TMZ with drugs targeting the PI3K/AKT/mTOR or immune checkpoint inhibitors (e.g., anti-PD-1/PD-L1) is being explored.

5. **Nanoparticle Delivery Systems**:

1. Nanocarriers for TMZ delivery can enhance drug accumulation at the tumor site, reducing systemic side effects and potentially overcoming resistance.

6. **Personalized Medicine Approaches**:

1. Genomic and transcriptomic profiling to identify patient-specific resistance mechanisms and tailor therapies.

7. **Immunotherapy**:

1. Approaches such as vaccines, CAR-T cells, and oncolytic viruses are being tested to address GBM resistance mechanisms.

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Current Research and Trials 1. **Biomarker Development**:

1. Identifying reliable biomarkers, such as MGMT promoter methylation or DDR pathway mutations, to predict TMZ response.

2. **Novel TMZ Derivatives**:

1. Designing TMZ analogs with improved potency and resistance profiles.

3. **Clinical Trials**:

1. Ongoing trials investigate various combination therapies, immunotherapies, and resistance modulators.

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Conclusion Understanding TMZ resistance in glioblastoma is critical to improving outcomes for patients with this devastating cancer. Advances in molecular biology, drug delivery systems, and personalized medicine hold promise for overcoming resistance and achieving better therapeutic efficacy.

In glioma, temozolomide resistance is due to overexpression of CD147 protein and induction of Nuclear factor-erythroid 2 related factor 2 [1)].

TMZ is currently the only mono-chemotherapeutic agent for newly-diagnosed high-grade glioma patients and acquired resistance inevitably occurs in the majority of such patients, further limiting treatment options. Therefore, there is an urgent need to better understand the underlying mechanisms involved in TMZ resistance, a critical step to developing effective, targeted treatments. An emerging body of evidence suggests the intimate involvement of a novel class of nucleic acid, microRNA (miRNA), in tumorigenesis and disease progression for a number of human malignancies, including primary brain tumors. miRNA are short, single-stranded, non-coding RNA (~22 nucleotides) that function as post-transcriptional regulators of gene expression [2)].

More studies are needed to elucidate the resistance mechanisms. In the current study, we investigated the relationship among the three important phenotypes, namely TMZ-resistance, cell shape and lipid metabolism, in GBM cells. We first observed the distinct difference in cell shapes between TMZ-sensitive (U87) and resistant (U87R) GBM cells. We then conducted NMR-based lipid metabolomics, which revealed a significant increase in cholesterol and fatty acid synthesis as well as lower lipid unsaturation in U87R cells. Consistent with the lipid changes, U87R cells exhibited significantly lower membrane fluidity. The transcriptomic analysis demonstrated that lipid synthesis pathways through SREBP were upregulated in U87R cells, which was confirmed at the protein level. Fatostatin, an SREBP inhibitor, and other lipid pathway inhibitors (C75, TOFA) exhibited similar or more potent inhibition on U87R cells compared to sensitive U87 cells. The lower lipid unsaturation ratio, membrane fluidity and higher fatostatin sensitivity were all recapitulated in patient-derived TMZ-resistant primary cells. The observed ternary relationship among cell shape, lipid composition, and TMZ-resistance may be applicable to other drug-resistance cases. SREBP and fatostatin are

suggested as a promising target-therapeutic agent pair for drug-resistant glioblastoma [3]

Gao et al. identified a lncRNA, PDIA3P1, which was upregulated in TMZ-resistant Glioblastoma cell lines. Overexpression of PDIA3P1 promoted the acquisition of Temozolomide resistance, whereas knockdown of PDIA3P1 restored TMZ sensitivity. PDIA3P1 was upregulated in MES-Glioblastoma, promoted PMT progression in GSCs, and caused Glioblastomas to be more resistant to TMZ treatment. Mechanistically, PDIA3P1 disrupted the C/EBPβ-MDM2 complex and stabilized the C/EBPβ protein by preventing MDM2-mediated ubiquitination. Expression of PDIA3P1 was upregulated in a time- and concentration-dependent manner in response to TMZ treatment, and TMZ-induced upregulation of PDIA3P1 was mediated by the p38α-MAPK signaling pathway. Neflamapimod is a small molecule drug that specifically targets p38α with excellent blood-brain barrier (BBB) permeability. NEF blocked TMZ-responsive PDIA3P1 upregulation and produced synergistic effects when combined with TMZ at specific concentrations. The combination of TMZ and NEF exhibited excellent synergistic antitumor effects both in vitro and in vivo [4].

At least 50% of TMZ treated patients do not respond to TMZ. This is due primarily to the over-expression of O6-methylguanine methyltransferase (MGMT) and/or lack of a DNA repair pathway in Glioblastoma cells. Multiple Glioblastoma cell lines are known to contain TMZ resistant cells and several acquired TMZ resistant Glioblastoma cell lines have been developed for use in experiments designed to define the mechanism of TMZ resistance and the testing of potential therapeutics. However, the characteristics of intrinsic and adaptive TMZ resistant Glioblastoma cells have not been systemically compared [5]

Many other molecular mechanisms have come to light in recent years. Key emerging mechanisms include the involvement of other DNA repair systems, aberrant signaling pathways, autophagy, epigenetic modifications, microRNAs, and extracellular vesicle production [6].

To date, aberrations in O6-methylguanine-DNA methyltransferase are the clear factor that determines drug susceptibility. Alterations of the other DNA damage repair genes such as DNA mismatch repair genes are also known to affect the drug effect. Together these genes have roles in the innate resistance, but are not sufficient for explaining the mechanism leading to acquired resistance. Recent identification of specific cellular subsets with features of stem-like cells may have role in this process. The glioma stem-like cells are known for its superior ability in withstanding the drug-induced cytotoxicity, and giving the chance to repopulate the tumor. The mechanism is complicated to administrate cellular protection, such as the enhancing ability against reactive oxygen species and altering energy metabolism, the important steps to survive [7].

Rabé et al. performed a longitudinal study, using a combination of mathematical models, RNA sequencing, single cell analyses, functional and drug assays in a human glioma cell line (U251). After

an initial response characterized by cell death induction, cells entered a transient state defined by slow growth, a distinct morphology and a shift of metabolism. Specific genes expression associated to this population revealed chromatin remodeling. Indeed, the histone deacetylase inhibitor trichostatin (TSA), specifically eliminated this population and thus prevented the appearance of fast growing TMZ-resistant cells. In conclusion, they identified in glioblastoma a population with tolerant-like features, which could constitute a therapeutic target [8)]

Ferroptosis, which is a new type of cell death discovered in recent years, has been reported to play an important role in tumor drug resistance. A study reviews the relationship between ferroptosis and glioma TMZ resistance, and highlights the role of ferroptosis in glioma TMZ resistance. Finally, the investigators discussed the future orientation for ferroptosis in glioma TMZ resistance, in order to promote the clinical use of ferroptosis induction in glioma treatment [9)].

CUL4B has been shown to be upregulated and promotes progression and chemoresistance in several cancer types. However, its regulatory effect and mechanisms on TMZ resistance have not been elucidated. The aim of this study was to decipher the role and mechanism of CUL4B in TMZ resistance. Western blot and public datasets analysis showed that CUL4B was upregulated in glioma specimens. CUL4B elevation positively correlated with advanced pathological stage, tumor recurrence, malignant molecular subtype and poor survival in glioma patients receiving TMZ treatment. CUL4B expression was correlated with TMZ resistance in Glioblastoma cell lines. Knocking down CUL4B restored TMZ sensitivity, while upregulation of CUL4B promoted TMZ resistance in Glioblastoma cells. By employing senescence β-galactosidase staining, quantitative reverse transcription PCR and Chromatin immunoprecipitation experiments, we found that CUL4B coordinated histone deacetylase (HDAC) to co-occupy the CDKN1A promoter and epigenetically silenced CDKN1A transcription, leading to attenuation of TMZ-induced senescence and rendering the Glioblastoma cells TMZ resistance. Collectively, our findings identify a novel mechanism by which Glioblastoma cells develop resistance to TMZ and suggest that CUL4B inhibition may be beneficial for overcoming resistance [10)].

CXCL12/CXCR4 has been demonstrated to be involved in cell proliferation, cell migration, cell invasion, angiogenesis, and radioresistance in glioblastoma (Glioblastoma). However, its role in TMZ resistance in Glioblastoma is unknown. Wang et al. aimed to evaluate the role of CXCL12/CXCR4 in mediating the TMZ resistance to Glioblastoma cells and explore the underlying mechanisms. They found that the CXCL12/CXCR4 axis enhanced TMZ resistance in Glioblastoma cells. Further study showed that CXCL12/CXCR4 conferred TMZ resistance and promoted the migration and invasion of Glioblastoma cells by up-regulating FOXM1. This resistance was partially reversed by suppressing CXCL12/CXCR4 and FOXM1 silencing. This study revealed the vital role of CXCL12/CXCR4 in mediating the resistance of Glioblastoma cells to TMZ, and suggested that targeting CXCL12/CXCR4 axis may attenuate the resistance to TMZ in Glioblastoma [11)].

The YTHDF2 expression in TMZ-resistant tissues and cells was detected. Kaplan-Meier analysis was employed to evaluate the prognostic value of YTHDF2 in Glioblastoma. Effect of YTHDF2 in TMZ resistance in Glioblastoma was explored via corresponding experiments. RNA sequence, FISH in

conjugation with fluorescent immunostaining, RNA immunoprecipitation, dual-luciferase reporter gene and immunofluorescence were applied to investigate the mechanism of YTHDF2 that boosted TMZ resistance in Glioblastoma.

YTHDF2 was up-regulated in TMZ-resistant tissues and cells, and patients with high expression of YTHDF2 showed lower survival rate than the patients with low expression of YTHDF2. The elevated YTHDF2 expression boosted TMZ resistance in Glioblastoma cells, and the decreased YTHDF2 expression enhanced TMZ sensitivity in TMZ-resistant Glioblastoma cells. Mechanically, YTHDF2 bound to the N6-methyladenosine (m6A) sites in the 3'UTR of EPHB3 and TNFAIP3 to decrease the mRNA stability. YTHDF2 activated the PI3K/Akt and NF-κB signals through inhibiting expression of EPHB3 and TNFAIP3, and the inhibition of the two pathways attenuated YTHDF2-mediated TMZ resistance.

YTHDF2 enhanced TMZ resistance in Glioblastoma by activation of the PI3K/Akt and NF-κB signalling pathways via inhibition of EPHB3 and TNFAIP3 [12].

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