

IN THE SPOTLIGHT

Understanding and Targeting Alkylator Resistance in Glioblastoma

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Summary: Alkylating chemotherapy is the mainstay in the treatment of pediatric and adult glioblastoma despite primary and acquired resistance and scientific efforts to precisely define therapies for individual patients. A focus on non-MGMT-mediated temozolomide resistance for pediatric glioblastoma suggests options for new drug combinations. *Cancer Discov*; 4(10); 1120-2. ©2014 AACR.

See related article by Agnihotri et al., p. 1198 (9).

In 2014, noncomplex, unspecific genotoxic therapies, specifically radiochemotherapy with the alkylating agent temozolomide, remain the therapeutic mainstay for most patients with a newly diagnosed glioblastoma. This is in sharp contrast to the rapid characterization of the cancer (epi)genome, paralleled by the development of drugs targeting oncogenically activated proteins that drive tumor growth. Apart from O⁶-methylguanine DNA methyltransferase (*MGMT*) promoter methylation, there are no predictive biomarkers allowing tailoring of primary therapies in adult glioblastoma. With the current tests, *MGMT* promoter hypermethylation signifying sensitivity toward alkylating agents occurs in one third of patients (1). Whether this rate is overestimated for pediatric glioblastoma or other mechanisms of chemoresistance are acting in this molecularly distinct disease is not well understood. Although data demonstrating a wide range of *MGMT* methylation stem from uncontrolled series, controlled trials with adequate *MGMT* analyses are not yet available.

Only recently has it become evident that *MGMT* activity is regulated by not only cell-intrinsic but also cell-extrinsic cues. In addition to promoter methylation, histone modifications, aberrant expression or function of transcriptional activators or repressors, and posttranscriptional regulation by various miRNA species can regulate *MGMT* expression. Increased methylation of histone H3 lysine 9 (H3K9) and concomitant binding of methyl-CpG-binding protein 2 (MeCP2), a methylated DNA-binding protein whose dysfunction leads to various neurodevelopmental disorders, to the *MGMT* promoter region were associated with promoter methylation and transcriptional downregulation, whereas histone H3 and H4 acetylation and methylation of H3 lysine 4 were detected in *MGMT*-expressing cells (2, 3). Increased acetylation of

H3K9 and decreased dimethylation of this residue have been linked to *MGMT* upregulation and acquired temozolomide resistance in glioblastoma cell lines. Treatment with histone deacetylase inhibitors potentiated the evolution of acquired temozolomide resistance (4). Induction of resistance may also be conferred by the hypoxia-, radiation-, and steroid-inducible mTOR downstream target N-myc downstream-regulated gene 1 (*NDRG1*), the protein product of which binds to and thus probably sustains action of *MGMT* (5).

Patients with no apparent expression of *MGMT* may also be resistant to temozolomide, but the mechanism underlying this resistance is incompletely understood. There is both clinical and experimental evidence that pediatric and adult glioblastoma differ in their responsiveness to temozolomide, with adult glioblastoma being more responsive than pediatric glioblastoma. However, pediatric glioma trials commonly lack determination of *MGMT* status, and a solid basis to relate temozolomide responsiveness to *MGMT* status is missing. In addition, there are robust accumulating data showing a molecular difference between glioblastoma in pediatric and young adult, and potentially also in elderly, patients. One third of pediatric glioblastoma is characterized by *H3F3A* mutations affecting two critical amino acids (K27 and G34) of histone H3.3, resulting in a separate epigenetic subgroup of glioblastoma that is distinct from *IDH1*-mutated glioblastoma (occurring mainly in younger adults) and the mesenchymal, receptor tyrosine kinase I and II subgroups that mainly comprise the older adult patients (6). As for the other gene expression-based subgroups, a predictive property or a novel therapeutic strategy has not yet emerged. Elderly patients commonly present with glioblastoma lacking all (epigenetic) positive prognostic markers (except the predictive *MGMT*; ref. 7).

Cellular responses to DNA damage involve distinct DNA-repair pathways, such as mismatch repair (MMR) and base excision repair (BER). MMR is critical for mediating the cytotoxic effect of O⁶-methylguanine. The MMR pathway consists of several proteins (hMLH1, hPMS2, hMSH2, hMSH3, and hMSH6) and corrects errors in DNA base pairing arising during DNA replication. During DNA replication, DNA polymerase mispairs O⁶-methylguanine with thymine, which triggers MMR-dependent removal of the mispaired thymine. However, the O⁶-methylguanine remains, and subsequent mispairing of O⁶-methylguanine with yet another

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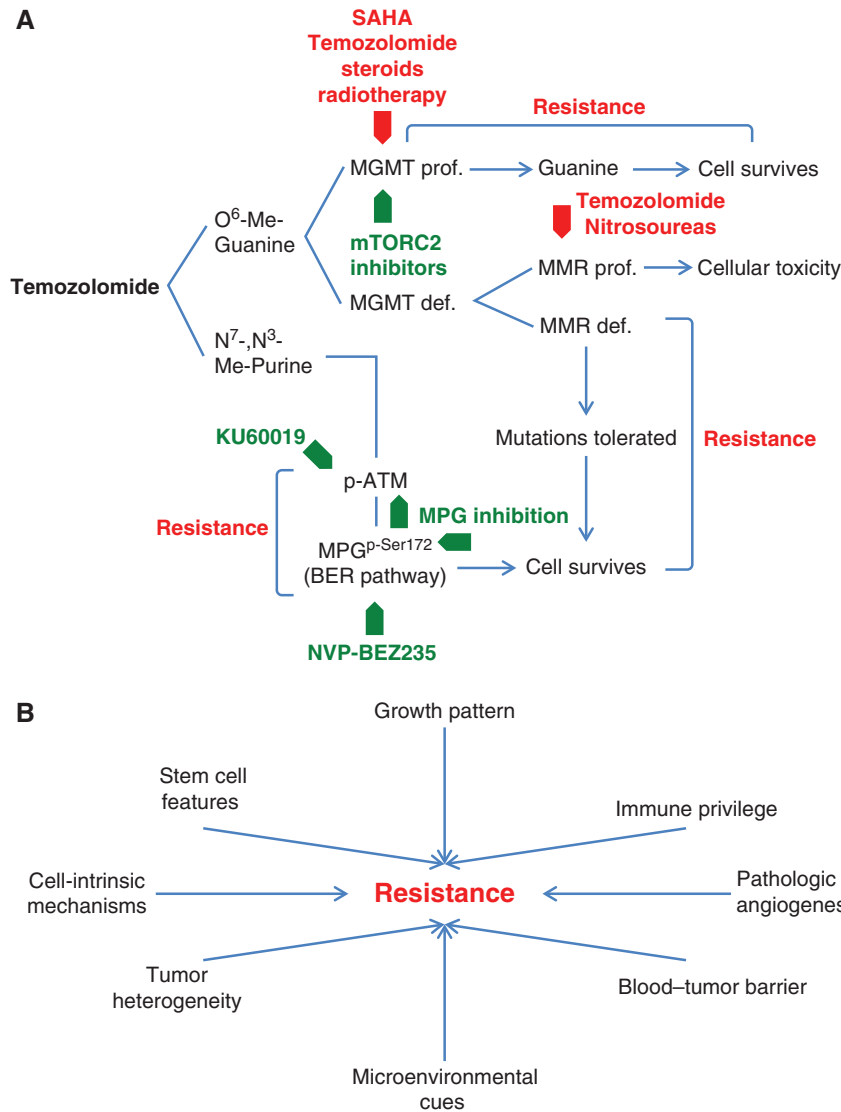


Figure 1. MGMT-dependent and MGMT-independent mechanisms of temozolomide resistance. **A**, factors enhancing resistance to temozolomide are depicted in red, and the respective arrowheads indicate the point of action. At the level of MGMT, molecular and iatrogenic factors enhancing resistance in MGMT-proficient glioblastoma cells [suberoylanilide hydroxamic acid (SAHA) is a histone deacetylase inhibitor] are depicted. In MMR-deficient cells, the cells may become tolerant to the mispairing of O⁶-methylguanine with thymine (mutations tolerated). In green print with the respective arrowheads showing the site of action, compounds to reduce resistance are provided. Preclinical data provide evidence that KU60019 and NVP-BEZ235 reduce resistance to alkylating chemotherapy (and radiotherapy) at the levels of ATM and MPG and other BER proteins, respectively. mTOR complex 2 (mTORC2) inhibition may reduce resistance to temozolomide and lomustine in MGMT-proficient cells. Resistance is limited to cell-intrinsic mechanisms, leading to limited efficacy of temozolomide, but not in a broader sense as in **B**. ATM, ataxia telangiectasia mutated; MPG, 3-methylpurine-DNA glycosylase. **B**, resistance features of glioblastoma beyond cell-intrinsic mechanisms that prevent toxicity from alkylating chemotherapy.

thymine leads to repetitive rounds of MMR (8). Defects in this system may cause resistance to temozolomide, as cells presumably become tolerant to the mispairing of O⁶-methylguanine with thymine. Although the majority of the temozolomide-induced lesions, N⁷-methylguanine and N³-methyladenine, are BER substrates, these DNA lesions are also readily repaired. Conceptually, blocking BER should enhance response to temozolomide.

In this issue of *Cancer Discovery*, Agnihotri and colleagues (9) hypothesized that alternate MGMT-independent mechanisms of DNA repair and treatment resistance could explain why pediatric glioblastoma fails to respond to alkylating agents. Using siRNA screening, multiple members of the BER pathway mediating alkylating agent resistance in pediatric glioblastoma were identified, including 3-methylpurine-DNA glycosylase (MPG). MPG is a DNA glycosylase responsible for initial recognition of the damaged DNA caused by temozolomide, specifically alkylated N⁷ guanine and alkylated N³ adenine residues. Previously, it had been proposed that unrepaired N³-methyladenine is toxic due to its ability to

directly block DNA polymerization. The MPG-mediated BER pathway promotes resistance to temozolomide in pediatric glioblastoma, and loss of MPG results in an accumulation of unrepaired cytotoxic N³-methyladenine residues, which results in curbed DNA replication and cell death.

Agnihotri and colleagues (9) also link the MPG-mediated BER pathway to ataxia telangiectasia mutated (ATM), which is central for cell-cycle checkpoints and double-strand DNA repair. ATM activity leads to direct phosphorylation of MPG at serine 172. By linking ATM signaling with MPG, there is an increased chance to identify novel targets that tumor cells use to repair damage caused by alkylating agents. Importantly, targeting BER and ATM increases sensitivity to temozolomide (and radiotherapy; ref. 10) and is preclinically effective in pediatric glioblastoma. ATM-related kinases are also a target of NVP-BEZ235, which inhibited both DNA-PKcs and ATM and attenuated the repair of ionizing radiation-induced DNA damage *in vivo* (11).

Previously, primary glioblastoma models in which p53 was defective and PI3K was expressed were sensitized to

radiotherapy by the ATM inhibitor KU60019 (10). In contrast, children with Li-Fraumeni syndrome caused by *TP53* germline mutations or Seckel syndrome induced by *ATM* and *ATR* mutations may not only not benefit from KU60019 or targeting MPG, but also experience unwanted cytotoxic effects on normal tissue. In patients without this type of germline mutation, the redundancy of DNA repair mechanisms in the ATM-p53-CHEK2 or BER pathways should prevent normal tissue toxicity. It is proposed that p53 proficiency may have opposite effects for chloroethylating nitrosoureas nimustine [1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea, ACNU], carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea, BCNU], or lomustine [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, CCNU]. These drugs may be more toxic for *TP53*-mutated glioma cells (12). By taking a broader, unsupervised view on the role of DNA-damage response pathways in pediatric glioblastoma, the present study allows novel approaches to overcome alkylating agent resistance and potentially also radioresistance. With these findings, the authors refocus on understanding and targeting resistance mechanisms for the most commonly used, and by far most effective, modalities for the treatment of glioblastoma (Fig. 1A). Until now, these preclinical hypotheses have not been challenged with clinical data, and alkylating chemotherapy is applied widely, in both pediatric and adult patients with glioblastoma despite cell-intrinsic resistance mechanisms being present that can be easily tested. Also, as a consequence of this dissociation between preclinical and clinical therapy development, there has been no relevant progress in outcome for patients with glioblastoma over the past 10 years.

More broadly, refinement of the definition of “resistance” is necessary (Fig. 1B). Resistance is often equated with drug resistance and focuses on intracellular mechanisms. Because of the lack of tissues at recurrence in most adult and pediatric glioblastomas and the lack of systematic evaluation of paired samples or biopsy-treat-biopsy approaches, most research but also clinical decisions are derived from surgically excised, primary, untreated tissues, and not tissues remaining in the brain responsive to the pressure of therapies and tumor-host interactions. More realistically, heterogeneity of tumor cells (13), interactions with the microenvironment, and angiogenic and immunologic statuses should be integrated, requiring new models and a closer relationship to the clinical outcome to pass the reality check and provide a unique opportunity to understand and ultimately overcome glioblastoma treatment resistance.

Disclosure of Potential Conflicts of Interest

W. Wick reports receiving commercial research support from Roche and Boehringer Ingelheim, has received honoraria from the

speakers' bureau of Prime Oncology, and is a consultant/advisory board member for Roche. No potential conflicts of interest were disclosed by the other author.

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