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# Targeting DNA double-strand break repair for glioblastoma chemotherapy

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## ABSTRACT

Glioblastoma Multiforme (GBM) is the most prevalent primary malignant brain tumor in adults. Despite improvements in surgery, irradiation, and chemotherapeutic treatments, GBM remains a clinically unresolved problem. We sum up how GBM is currently treated, with a focus on temozolomide (TMZ) and chloronitrosoureas. We condense how such agents evoke lethal DNA damage in transformed cells and how these counteract such mechanisms. A better knowledge of such pathways may pave the way for improved therapies. Therefore, we recapitulate how inhibitors of the DNA repair factors PARP and RAD51 as well as epigenetic modulators of the histone deacetylase (HDAC) family might be useful in combination with established methylating and alkylating agents against GBM.

## KEYWORDS

Alkylating agents, DNA damage, HDAC, Glioblastoma, PARP, RAD51

## BACKGROUND

GBM is known for its aggressive progression, weak response to cancer therapy and, consequently, bad prognosis [1,2]. A poor 5-year overall survival rate of less than 10% points to the need for new chemotherapeutic approaches. The current standard care of GBM includes maximal surgical resection, followed by radiation and adjuvant chemotherapy with the methylating agent temozolomide [2-4]. Chloroethylating nitrosoureas (chloronitrosoureas, including lomustine, nimustine, carmustine, and fotemustine) are also used as first- and second-line chemotherapeutics for the treatment of GBM and other brain tumors or metastases of various origins [5,6].

Temozolomide and chloronitrosoureas induce pre-toxic DNA lesions (adducts) which lead to cancer cell death [6]. Temozolomide modifies DNA bases by alkylation reactions on N- or O-atoms. One of the most critical lesions is the minor adduct $O^6$-methylaguanine ($O^6$-MeG), because of its potent genotoxic and cytotoxic effects. It can be repaired in a one-step reaction by the $O^6$-methylguanine-DNA Methyltransferase (MGMT). MGMT is the first line of defence against $O^6$-alkylation damage [7]. In cells lacking MGMT activity, backup mechanisms operate to repair or remove the damaged bases, such as the Mismatch Repair (MMR) system.

During DNA replication in S phase, DNA polymerases mismatch $O^6$-MeG with thymine. The MMR system recognizes this and removes the thymidine. However, this repair is futile, since the mispairing is repeated [8]. These unsuccessful attempts of MMR to repair the mismatches lead to an accumulation of long-lasting single-stranded DNA segments which are further transferred into critical DNA double-strand breaks (DSB) in the S-phase of the post-treatment cell cycle [9].

Similar to methylating agents, the chloronitrosoureas induce a broad spectrum of DNA adducts. Among these, $O^6$-chloroethylguanine ($O^6$-ClEG) is suggested to be the main cytotoxic lesion. This adduct is unstable and undergoes intramolecular rearrangement that lead to an intermediate, N1-$O^6$-ethenoguanine. This is converted during a second intramolecular rearrangement to a N1-guanine-N3-cytosine interstrand cross-link (ICL) [6]. Similar to $O^6$-MeG, $O^6$-ClEG is a substrate for MGMT. Replicating cells lacking MGMT activity develop a complex backup mechanism for ICL repair that leads to the generation of DSBs during the removal of ICL by Nucleotide Excision Repair (NER) proteins [6,10].

The processing of both $O^6$-MeG and $O^6$-ClEG-derived ICLs generate DSBs, which represent lethal secondary DNA damage. These DSBs are substrates for DNA repair by the non-homologous end-joining (NHEJ) and homologous recombination (HR) [11]. The canonical NHEJ (C-NHEJ),

with its key proteins KU70/KU80 and the catalytic subunit of the DNA damage-sensing checkpoint kinase DNA protein kinase (DNA-PK), is functional throughout the cell cycle. NHEJ is most important in the G1 phase, where HR is lacking due to a lack of a homologous DNA strand. However, C-NHEJ plays only a minor role in the repair of $O^6$-MeG and $O^6$-ClEG induced DNA replication-dependent DSBs [12,13]. In addition to C-NHEJ, another DSB repair pathway is described, the backup NHEJ (B-NHEJ). It depends on the enzymatic activities of PARP1 (poly(ADP-ribose)-polymerase) 1, ligase III, and X-ray Repair Cross-Complementing Protein 1 (XRCC1) [14]. PARP1 recognizes DSBs, whereupon it modifies itself and proteins in the surrounding chromatin. During this process termed PARylation, PARP1 adds Poly-ADP-Ribose (PAR) chains to histones and non-histone proteins [15]. The presence of the PAR binding motif PBM in both B-NHEJ proteins like XRCC1 and ligase III, or C-NHEJ proteins like Ku70 and DNA-PK indicates that PARP activity is required for their recruitment to DSBs [16].

The second main DNA DSB repair pathway, HR, is operative in the late S and G2 phases of the cell cycle. HR involves strand invasion onto the sister chromatid template, followed by reparative DNA synthesis, and resolution of Holiday junctions [17]. Usage of an undamaged template ensures error-free DSB repair. Due to its recombinase activity RAD51 is the key HR protein. RAD51 overexpression has been observed by immunohistochemistry in various cancers [18-23], including gliomas [24]. In most studies, RAD51 overexpression was associated with poor prognosis for the patients. Owing to its important role in the repair of temozolomide- and chloronitrosoureas-induced DNA damage, HR is considered as an emerging target for glioblastoma therapy [25-27].

A recent study with a mouse orthotopic implantation model of human patient-derived glioblastoma cells corroborated that, besides MGMT expression, the MMR, NER, and HR contribute to temozolomide resistance. Importantly, this determines the survival of tumor-bearing mice [28]. Especially gliomas without MGMT activity rely on HR as major DNA DSB repair pathway. In light of these findings, it is relevant that high throughput screening revealed several small molecules as selective inhibitors of RAD51 [29-31]. Remarkably, such agents are able to suppress the growth of breast cancer cells [32,33] and glioblastoma cells [34] *in vitro and in vivo* (Figure 1).

Whereas HR has been so far targeted only in preclinical investigations, PARP enzymes are well-established targets in chemotherapy. In glioblastoma *in vitro* and in xenografts, the combined treatment with temozolomide and the pharmacological PARP inhibitors (PARPi) rucaparib or veliparib showed superior efficacy over single temozolomide treatment [35-40] (Figure 1). Because PARP1 is involved in the B- and C-NHEJ mechanisms for repair of DSB in the absence of functional HR [25-27], HR defective cells, and specifically those with BRCA2 deficiency, show hypersensitivity towards PARPi [41,42].

This augmented susceptibility of BRCA2 mutant cells resembles the phenomenon "synthetic lethality", a term that describes the lethal gene interactions of two defective cellular pathways [43]. Synthetic lethality is of clinical interest, since it allows a genetically based stratification of patients into effective therapies. "Synthetic lethality"-like effects can be expected if PARPi are combined with RAD51 small molecule inhibitors.

The interplay between PARP activity and other DSB repair mechanisms is the subject of intense research [44-46]. A disadvantage in the use of PARPi for glioblastoma therapy is the fact that established PARPi, like olaparib and rucaparib, are substrates of P-glycoproteins. This efflux system removes them rapidly from cells, reduces their uptake through the brain-blood barrier, and thereby their efficiency to kill glioblastoma cells [37,47]. In order to increase their pharmacological applicability, combinations with drugs that act as efflux pump inhibitors are investigated in cancer cell models [48] including in glioblastoma [49].

Another implementable strategy is the downregulation of RAD51 or other essential HR players by inhibition of proteins involved in their regulation. For example, multiple studies have reported that class I HDACs, HDAC1, HDAC2, HDAC3, and HDAC8, promote the expression of HR proteins (Fig. 1). Thus, these epigenetic enzymes are increasingly appreciated targets for which more and more clinically approved HDAC inhibitors are developed (for a detailed review [10]).

## CONCLUDING REMARKS

Due to the highly invasive phenotype of GBM and its ability to spread throughout the brain parenchyma, complete or near complete surgical resection is almost impossible. Consequently, the whereabouts of a residual tumor tissue after the operation is practically unavoidable. Despite the following aggressive radio-/chemotherapy, survival rates are usually inadequate due to development of resistance. This process frequently involves an

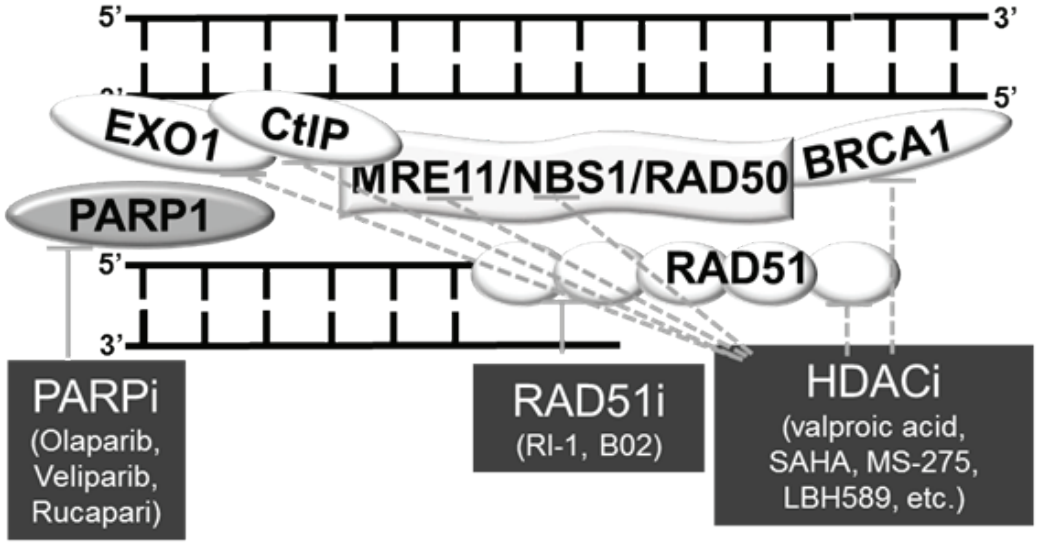


**Figure 1:** Effects of innovative inhibitors on HR proteins. RAD51 inhibitors RI-1 and B02 inhibit the DNA binding activity of the RAD51 recombinase and joint molecule formation during HR.
Various HDACi decrease the expression of RAD51 and downregulate proteins of the MRN complex, EXO1 or CtIP, or BRCA1, which mediate the repair of cytotoxic DNA lesions.

overexpression of DNA repair proteins and an ensuing activation of DNA repair pathways. DNA repair pathways protect glioblastoma cells from lethal DSBs that are induced by alkylating chemotherapeutics and serve as a second or third line of cellular defense. Among these DNA repair mechanisms, HR and PARP-dependent B-NHEJ are particularly important for glioblastoma resistance to chemotherapy in the absence of MGMT. In this way they represent suitable targets for inhibition/downregulation by small molecule inhibitors. There is at least one clinical trial designed to test a PARPi (olaparib) in combination with temozolomide and/or radiotherapy for treatment of patients with GBM [50]. Novel approaches utilizing PARPi, RAD51i or epigenetic drugs like some HDACi, which cause downregulation of HR proteins [10, 51], may overcome glioblastoma cell resistance to improve patient survival.

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