communications biology

A Nature Portfolio journal

Review article

https://doi.org/10.1038/s42003-026-10467-7

Molecular insights into DNA damage response plasticity in glioma stem cells

Nathan Christian Molinari[1,3], Valeria Lusi[1,3], Andrea Bianconi[1,2], Daniele De Pasquale[1], Debora Giunti[1], Pietro Fiaschi[1,2] & Elena Cerutti[1]

High-grade gliomas frequently recur after radiotherapy and temozolomide because tumor cells can adapt to sustained DNA damage. This Review discusses how glioma stem cells contribute to this resistance by rewiring the DNA damage response rather than simply increasing DNA repair efficiency. We examine how checkpoint signaling, replication stress tolerance, DNA repair pathway choice, chromatin and RNA-based regulation, translational control, metabolism and microenvironmental cues cooperate to preserve survival under genotoxic stress. By integrating these mechanisms, we define DNA damage response plasticity as an adaptive network that supports tumor persistence and recurrence, while exposing vulnerabilities that may guide more effective therapeutic strategies.

High-grade gliomas (HGGs) are the most aggressive gliomas of the central nervous system and remain associated with an extremely poor prognosis despite advances in diagnosis and therapy[1,2]. Their infiltrative growth, molecular heterogeneity, and plasticity render HGGs among the most challenging solid tumors to treat. Surgical resection remains the cornerstone of treatment, but standard Stupp-protocol therapy provides only modest survival benefit, with median overall survival around 15 months and <5% five-year survival[3]. In the recurrent setting, available options extend survival by only 6–9 months on average[4].

To better capture glioma heterogeneity, adult diffuse glioma classification has shifted from histopathological to integrated histo-molecular frameworks[5]. In the current WHO Classification of Tumors of the Central Nervous System, gliomas are stratified by combinations of molecular alterations, most notably *IDH* mutation status and 1p/19q codeletion, with additional alterations refining grading and diagnosis (Table 1)[5,6]. Although diagnostically essential, this classification does not fully capture the heterogeneity introduced by adaptive cellular states and plasticity. Although stem-like tumor-propagating populations occur across diffuse gliomas, the strongest mechanistic evidence linking GSCs to therapy resistance and DDR plasticity derives from IDH-wildtype glioblastoma, which therefore represents the primary focus of this Review.

Similarly, molecular markers, such as *MGMT* promoter methylation, *TERT* promoter mutations, and *EGFR* amplification contribute to prognostic and therapeutic stratification, but do not explain the adaptive mechanisms sustaining therapy resistance, particularly in *IDH*-wildtype HGGs (Table 2)[7,8].

These limitations have intensified interest in alternative therapeutic strategies, including immunotherapy and molecularly targeted approaches[9], alongside large-scale molecular profiling efforts that have uncovered recurrent genetic alterations, deregulated signaling pathways, and distinct cellular states driving tumor progression and treatment failure[10]. Among these, glioma stem cells (GSCs) have emerged as a key tumor-maintaining cellular state characterized by self-renewal capacity, lineage plasticity, and exceptional resistance to genotoxic stress induced by radiotherapy and chemotherapy. Importantly, GSCs represent a dynamic and inducible cellular state that can be regenerated from more differentiated tumor cells under microenvironmental and therapeutic pressure. A defining hallmark of GSCs is extensive rewiring of the DNA damage response (DDR). Rather than simply enhancing DNA repair efficiency, DDR plasticity involves dynamic regulation of checkpoint control, replication stress tolerance, repair pathway usage, chromatin and RNA-based regulation, metabolism, translation, and niche signaling to sustain survival under genotoxic stress. Accordingly, adaptive DDR states support not only lesion processing but also damage tolerance and survival-oriented cell-fate decisions.

In this review, we synthesize current evidence illustrating how GSCs exploit multilayered DDR networks to sustain survival, self-renewal, and therapy resistance in HGGs. By integrating checkpoint signaling, replication stress management, DNA repair pathway choice, RNA processing, epigenetic regulation, translational control, metabolism, and microenvironmental interactions, we outline the molecular logic of DDR plasticity in GSCs and highlight vulnerabilities that may inform mechanism-driven therapies. We also highlight transcriptional recovery after damage as a potential additional dimension of DDR fitness in highly transcriptional GSC states.

Current DNA repair-targeting therapies

Current standard-of-care treatment for HGGs is largely based on DNA-damaging strategies. Radiotherapy primarily induces DNA double-strand

[1]IRCCS Azienda Ospedaliera Metropolitana, Genoa, Italy. [2]Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy. [3]These authors contributed equally: Nathan Christian Molinari, Valeria Lusi. e-mail: elena.cerutti@aomliguria.it

Table 1 | WHO 2021 histo-molecular classification of adult diffuse gliomas

Tumor entity	Defining molecular features	Diagnostic role	Clinical relevance
Astrocytoma, *IDH*-mutant	*IDH1/IDH2* mutation; absence of 1p/19q codeletion; frequent *ATRX* loss and *TP53* mutation; *CDKN2A/B* homozygous deletion defines grade 4	Defines astrocytic lineage; molecular grading overrides histology	Better OS than *IDH*-wt GBM; *CDKN2A/B* deletion confers poor prognosis
Oligodendroglioma, *IDH*-mutant, 1p/19q-codeleted	*IDH* mutation + whole-arm 1p/19q codeletion; frequent *TERT* promoter mutation	Mandatory molecular criteria for diagnosis	Most favorable prognosis; high chemosensitivity
Glioblastoma, *IDH*-wildtype	*IDH*-wt plus ≥1 of: *TERT* promoter mutation, *EGFR* amplification, +7/ − 10 signature; frequent *CDKN2A/B* loss	Defines GBM even without classic histology	Poorest prognosis; median OS ~ 15 months

This table summarizes the WHO 2021 histo-molecular entities of adult diffuse gliomas, defined by mandatory combinations of genetic alterations rather than single biomarkers. Astrocytoma *IDH*-mutant, oligodendroglioma *IDH*-mutant and 1p/19q-codeleted, and glioblastoma *IDH*-wildtype represent distinct tumor entities with characteristic molecular profiles that refine diagnosis, grading, and clinical stratification beyond histology alone. These integrated molecular criteria form the basis of the current CNS5 classification and provide the structural framework for glioma taxonomy. *GBM* glioblastoma, *OS* overall survival.

Table 2 | Molecular markers with prognostic and predictive value and relevance to treatment response in HGGs

Molecular marker	Molecular alteration	Role/relevance to GSC-associated resistance	Clinical significance
***MGMT* promoter methylation**	Epigenetic silencing of *MGMT*	Predictive biomarker; in GSCs, association with TMZ sensitivity is stronger under differentiation-promoting than stem-like conditions[241]	Improved response to TMZ and longer OS
***TERT* promoter mutation**	C228T/C250T mutations	Supportive diagnostic/ prognostic marker; associated with sphere-forming capacity, tumorigenic potential, and stemness-linked drug resistance[242]	Worse prognosis, especially in *IDH*-wt gliomas
***EGFR* amplification**	Focal amplification of the *EGFR* locus	Driver alteration in *IDH*-wildtype GBM; linked to stem-like signaling programs and tumor-propagating glioblastoma cell states[243]	Common alteration in glioblastoma *IDH*-wildtype; relevant for biological stratification and targeted-therapy rationale

This table lists key molecular alterations that modulate prognosis, therapeutic response, or molecular grading in high-grade gliomas, but do not define independent histo-molecular tumor entities. Markers such as *MGMT* promoter methylation, *TERT* promoter mutations, and *EGFR* amplification contribute to risk stratification, prediction of treatment sensitivity, and refinement of tumor aggressiveness within WHO-defined glioma categories. In addition, where supported by available evidence, these markers may also intersect with stem-like, therapy-tolerant glioma cell states, further highlighting the complexity of glioma biology beyond entity-level classification. *TMZ* temozolomide, *OS* overall survival, *GBM* glioblastoma.

breaks and oxidative base damage, whereas TMZ introduces methyl adducts at several DNA positions, including N7-guanine, O3-adenine, and the clinically most relevant O6-guanine[11]. TMZ cytotoxicity depends on unsuccessful lesion processing, leading to replication stress, strand break accumulation, and apoptosis[12]. However, resistance to TMZ frequently arises through enhanced DNA repair, damage tolerance, or apoptosis suppression[13,14]. In the recurrent setting, additional alkylating agents such as lomustine and procarbazine are commonly used, either alone or in combination regimens such as PCV (Procarbazine, Lomustine, and Vincristine). Lomustine, a highly lipophilic chloroethylating agent, readily crosses the blood-brain barrier and induces DNA and RNA alkylation as well as interstrand cross-links at the O6 position of guanine, while procarbazine similarly converges on O6-guanine methylation. Their efficacy is likewise limited by the ability of glioma cells to tolerate and repair alkylation-induced lesions[15].

Together, these observations indicate that clinical failure is driven not by insufficient DNA damage induction, but by adaptive DNA damage response and repair programs shaped by clinically relevant molecular markers (Table 2). Importantly, this resilience is enriched within specific tumor cells subpopulations displaying heightened survival plasticity. In this context, glioma stem cells have emerged as central drivers of therapy resistance and tumor recurrence, explaining why DNA-damaging treatments fail to achieve durable disease control.

Glioma stem cells

GSCs are recognized as key drivers of glioma initiation, progression, therapy resistance, and recurrence[16]. Early studies identified tumor-initiating populations by enriching for $CD133^{+}$ cells; however, $CD133^{-}$ fractions can also retain tumor-initiating capacity, indicating that stemness cannot be defined by a single marker[17]. Accordingly, multiple surface markers have been associated with GSC populations, including CD44[18], CD15[19], A2B5[20], CD90[21], integrin α6[22], CD171/L1CAM[23], and EpCAM, whose overexpression identifies highly tumorigenic and therapy-resistant GBM subsets[24]. Beyond surface phenotypes, stem-like states are defined by transcriptional and epigenetic programs linked to neural progenitor identity and self-renewal, including *BMI1*, *SOX2*, *MSI1/2*[25], *NANOG*[26], and *Nestin*[27], as well as YAP/TAZ signaling[28]. Stemness is also highly plastic: *OCT4* reactivation in therapy-adapted GBM cells promotes stem-like programs, metabolic rewiring, and stress survival, highlighting the reversibility of the GSC state[29]. Consistently, a core transcriptional network composed of *OCT4*, *SOX2*, *SALL2*, and *OLIG2* is sufficient to reprogram differentiated GBM cells into induced GSCs that retain self-renewal, tumor-initiating capacity, and therapy resistance[30]. In vivo, GSCs are enriched in anatomical niches such as the subventricular zone, where niche-derived signals support stemness, therapy resistance, and recurrence[31].

Together, these findings indicate that GSCs represent a dynamic and plastic state whose adaptability underlies their capacity to survive genotoxic stress, setting the stage for DDR-centered mechanisms of therapy resistance.

Clinical and biological basis of therapy resistance in GSCs

Long before GSCs were molecularly defined, clinical observations suggested that therapeutic response in malignant gliomas could not be explained solely by histology, tumor size, anatomical location, or treatment intensity. In a seminal study, Rosenblum et al. showed that younger patients had significantly longer post-operative survival than older patients despite comparable clinical characteristics, attributing this difference to increased intrinsic chemosensitivity of clonogenic tumor cells to the nitrosourea BCNU rather than to reduced tumor aggressiveness[32]. Importantly, survival correlated inversely with in vitro clonogenic survival following chemotherapy.

These findings provided early evidence that therapy resistance is encoded within specific tumor cells subpopulations, anticipating the later

identification of stem-like, therapy-resistant compartments in HGGs. GSCs can therefore be viewed as the cellular basis of clinically observed heterogeneity in treatment response and disease progression. Subsequent studies showed that GSC resilience reflects not simply enhanced DNA repair, but a highly plastic DDR state in which checkpoint control, replication stress tolerance, and cell fate decisions are rewired to sustain survival under genotoxic stress.

Together, these interconnected mechanisms define a multilayered and adaptive DNA damage response framework underlying therapy resistance in GSCs (Fig. 1). Accordingly, DDR plasticity emerges as a defining hallmark of GSCs and a central driver of therapy resistance in HGG. Recent single-cell and spatial transcriptomic studies further revealed that glioblastoma is composed of dynamic malignant cellular states and spatially organized ecosystems rather than a single fixed cellular hierarchy[33,34]. Although not specific to GSCs, these findings support a model in which stem-like tumor-maintaining states are heterogeneous, inducible, and context-dependent. Consistently, GSC-focused studies show that stem-like cells occupy distinct transcriptional states and niches, suggesting that DDR plasticity is shaped by transcriptional identity, microenvironmental cues, and therapeutic pressure[35,36]. Accordingly, the mechanisms discussed below are organized into two complementary levels: core checkpoint and DNA repair pathways, and broader regulatory layers sustaining adaptive DDR competence in GSCs. Some signaling nodes, therefore, recur across sections as they are redeployed in distinct biological contexts. The following sections dissect these mechanisms, with supporting experimental models summarized in Supplementary Table 1. Details of the literature search strategy and the PRISMA flow diagram (Supplementary Fig. 1) are provided in the Supplementary Information.

Checkpoint signaling and replication stress

GSCs are increasingly recognized as a tumor-propagating state sustained by reinforced DDR signaling and hyperactive cell-cycle checkpoints, forming a central axis of radio- and chemoresistance. Seminal studies showed that, following irradiation, GSCs rapidly and persistently activate the ATM/ATR-Chk1/Chk2 cascades, enforcing a robust G2/M checkpoint and resolving DNA double-strand breaks (DSBs) more effectively than differentiated glioma cells. This phenotype reflects checkpoint hyperactivation and replication slowing rather than intrinsically superior repair capacity, enabling prolonged damage tolerance and delayed cell-cycle transitions[37–42]. Pharmacologic ATM inhibition restores sensitivity to DSB-inducing agents, supporting checkpoint reinforcement as central to GSC survival[43].

A key driver of this state is chronic intrinsic replication stress. GSCs exhibit reduced fork velocity, γH2AX/53BP1 accumulation at replication sites, and elevated RNA-DNA hybrids driven by transcription of long neural genes. Rather than inducing fork collapse, this stress sustains ATR-Chk1 signaling and checkpoint addiction, conferring radioresistance but exposing a vulnerability, as combined ATR/PARP inhibition abolishes self-renewal in vitro and in vivo[41,44,45]. CDK12/CDK13 inhibition similarly disrupts transcriptional elongation and replication fork progression in glioblastoma, linking transcription-replication conflict to checkpoint-dependent

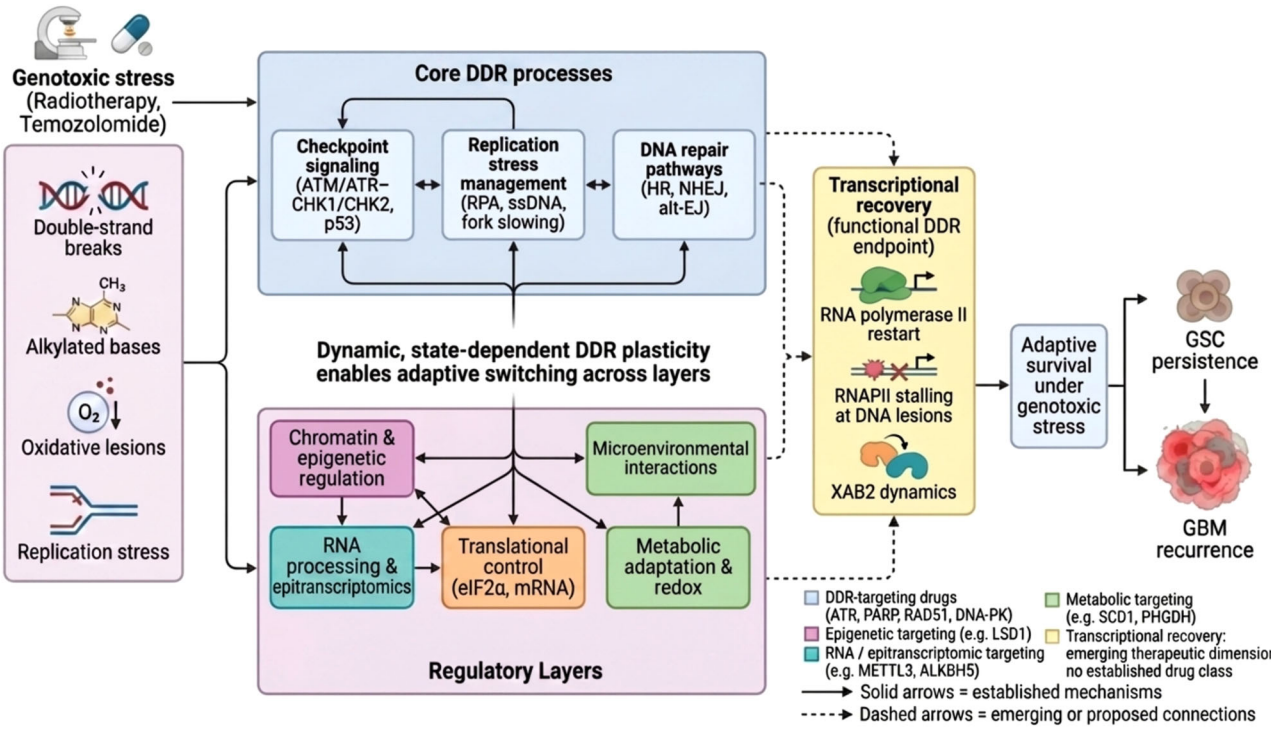

Fig. 1 | Multilayered DDR plasticity in glioma stem cells and therapeutic vulnerabilities. Genotoxic stress induced by radiotherapy and temozolomide generates diverse DNA lesions, including double-strand breaks, alkylated bases, oxidative damage, and replication stress. These insults activate core DNA damage response (DDR) processes, including checkpoint signaling, replication stress management, and the selection of DNA repair pathways. In glioma stem cells (GSCs), these processes are embedded within a broader network of regulatory layers comprising chromatin and epigenetic regulation, RNA processing and epitranscriptomic control, translational adaptation, metabolic rewiring, and microenvironmental interactions. Dynamic, state-dependent DDR plasticity enables adaptive switching across these layers, promoting survival under genotoxic stress. Transcriptional recovery, defined as restoration of RNA polymerase II activity following damage, is highlighted as a functional DDR endpoint and an emerging determinant of therapy resistance. Color-coded modules indicate major therapeutically targetable axes, while solid arrows denote experimentally established mechanisms and dashed arrows represent emerging or proposed connections. This integrated network ultimately sustains GSC persistence and drives glioblastoma recurrence. Blue modules indicate DDR-targeting strategies (e.g., ATR, PARP, RAD51, DNA-PK inhibitors), pink modules indicate epigenetic targeting approaches (e.g., LSD1 inhibition), cyan modules represent RNA/epitranscriptomic targeting (e.g., METTL3, ALKBH5), green modules indicate metabolic targeting strategies (e.g., SCD1, PHGDH), and yellow modules represent transcriptional recovery as an emerging therapeutic dimension without an established drug class. Solid arrows denote experimentally established mechanisms, whereas dashed arrows indicate emerging or proposed functional connections. Created in BioRender. Cerutti, E. (2026) https://BioRender.com/7fjatyc.

survival[46], while PARG inhibition in glioma stem cells induces replication arrest, intra-S-phase checkpoint activation, and apoptosis in an NAD + -dependent manner, identifying replication stress buffering as an actionable dependency[47].

Multiple buffering systems further support tolerance to replication-associated damage. Telomere integrity represents a major DDR node: the G-quadruplex ligand telomestatin induces telomeric dysfunction in GSCs, triggering ATR-Chk1 activation and replication-dependent death while sparing non-stem tumor cells[48]. Resolution of replication-associated topological stress relies on topoisomerase IIβ (TOP2β); its depletion sensitizes GSCs to TMZ and alkylating stress, shifting their response toward differentiated cells[49]. Replication protein A (RPA) overexpression likewise supports GSC survival, and its inhibition causes DSB accumulation and radiosensitization, highlighting dependence on fork protection rather than lesion removal[50]. Additional work indicates that checkpoint protection is modulated by upstream signaling, with IGF1R promoting radioresistance[51] and CDC20 perturbation increasing therapy sensitivity[52], further linking checkpoint function to broader survival and cell-cycle networks.

Within this checkpoint-dominated landscape, canonical repair pathways are temporally reshaped to sustain damage tolerance. Delayed Fanconi anemia pathway activation and differential reliance on homologous recombination (HR), including RAD51 and FA components, permit prolonged G2 arrest and survival with unresolved lesions[44,53,54]. Telomere-associated DDR further reinforces this state: ALT-positive GSCs maintain chronic telomeric damage signaling while preserving chromosomal stability, supporting long-term persistence[55,56]. Preclinical studies in 2D and 3D GSC models support the therapeutic relevance of this checkpoint-addicted state[57], while altered radiation delivery, including extra-high-dose-rate irradiation[58], further supports context-dependent of checkpoint robustness.

Collectively, these studies define GSC resistance as a replication-stress-driven, checkpoint-addicted DDR state, critically dependent on sustained ATR/Chk1 signaling, fork protection, telomere integrity, and delayed engagement of repair pathways. Checkpoint reinforcement should therefore be viewed less as a marker of superior repair than as a mechanism of damage tolerance preserving GSC survival under sustained genotoxic stress.

DDR plasticity and adaptive survival programs

Beyond checkpoint addiction and replication-stress dependence, GSCs exhibit profound plasticity in DDR outputs. DNA damage sensing remains intact, whereas checkpoint enforcement, apoptosis, and differentiation are selectively attenuated, converting DDR into a permissive survival program shaped by oncogenic, inflammatory, niche-derived, and therapy-induced signals[59,60].

Autocrine niche-like signaling is a major mechanism for modulating DDR. The EDN3-EDNRB axis maintains undifferentiated, anti-apoptotic and clonogenic GSC states under stress; its disruption abolishes self-renewal and tumorigenicity, indicating that survival-oriented DDR tuning sustains recurrence[61]. Inflammatory cues further decouple DDR activation from tumor suppression: prolonged IL-1β induces oxidative DNA damage and DDR signaling, while COX-2 suppresses p53, disabling apoptosis and checkpoint arrest under chronic genotoxic stress[62]. Periarteriolar and engineered perivascular niches similarly reinforce GSC enrichment and radioresistance, indicating niche-dependent stabilization of adaptive DDR states[63,64]. Laminin α2-dependent adhesion likewise supports stem-cell maintenance and stress tolerance[65].

Oncogenic and adhesion-dependent pathways further tune DDR output. L1CAM links invasive traits to nuclear genome surveillance by regulating the MRN-ATM-Chk2 axis via NBS1[66]. MET-high GSCs exhibit elevated ATM, Chk2, and RAD51 activation together with cytoplasmic anti-apoptotic p21; MET inhibition impairs DSB repair and restores radiosensitivity[67]. Integrin α6 likewise becomes essential after fractionated radiotherapy, supporting DSB repair and survival[68]. Distinct TMZ exposure conditions also drive different resistant states, indicating that DDR plasticity is dosage-dependent[59].

Mitotic control, metabolic state, and post-translational regulation further reinforce checkpoint permissiveness. MELK sustains chromosomal integrity through FOXM1-Aurora B and ATM/ATR signaling, and its inhibition induces mitotic catastrophe and radiosensitization[69]. PDK1 cooperates with Chk1 to maintain G2/M control, and dual targeting, phenocopied by UCN-01, triggers catastrophic checkpoint collapse[70]. Chloroquine likewise underscores the context dependence of this balance, as it concurrently activates pro-survival and death-inducing signaling in GSCs[71].

Metabolic support of DDR is also critical: elevated glycolysis sustains checkpoint and HR signaling, whereas GAPDH inhibition disrupts ATM/Chk activation and induces genomic fragmentation[72]. The deubiquitinase USP1 stabilizes ID1 and CHEK1, sustaining stem-like identity and checkpoint signaling; USP1 inhibition enhances radiation-induced DNA damage and improves survival in vivo[53]. Likewise, PAF/KIAA0101 promotes translesion synthesis (TLS) and radioresistant self-renewal; its depletion or TLS inhibition reduces sphere formation and sensitizes cells to irradiation[73].

These adaptive DDR states are increasingly exploited therapeutically. ATR inhibition synergizes with temozolomide in *MGMT*-methylated cells, increasing γH2AX, apoptosis, and clonogenic loss[74]. The cancer-testis lncRNA *PITAR* promotes adaptive rewiring by stabilizing TRIM28 and promoting p53 degradation, whereas *PITAR* knockdown restores p53 signaling and sensitizes cells to TMZ[75]. Multi-target approaches reinforce this concept: the brain-penetrant Hsp90 inhibitor NXD30001 suppresses self-renewal while attenuating DDR and ER stress and synergizes with radiotherapy[76], whereas arsenic trioxide combined with (-)-gossypol downregulates DDR genes and selectively kills GSCs ex vivo[77]. The natural product taccaoside A similarly targets DDR-supporting oncogenic signaling by inhibiting HRAS/KRAS-PI3K-AKT and MAPK-ERK pathways, inducing apoptosis and loss of stemness in vivo[78]. Additional approaches, including nitric oxide, PBI-05204, and oHSV-P10, similarly destabilize adaptive survival states without directly targeting repair enzymes[79–81].

Radiobiological context further shapes DDR plasticity. Total radiation dose, rather than dose rate, governs response[58], while proton beams outperform photons by inducing overwhelming ROS and DDR collapse[82]. Targeted radio-pharmacologic strategies can override quiescence: Sonic Hedgehog activation forces dormant cells into S phase, enabling [I-125] ITdU incorporation and selective elimination of GSC populations[83]. Biological therapies directly exploit DDR rewiring: oHSV-G47Δ with TMZ sequesters ATM and suppresses ATR signaling[84], oHSV-TRAIL induces potent apoptosis in recurrent tumors[85], and parvovirus MVM preferentially replicates in p53-altered GSCs, triggering DDR-dependent bystander damage in vivo[86]. Inhibition of Src signaling with AZD0530 further radiosensitizes glioma cells by blocking radiation-induced Src activation[87]. Advanced tumor models further underscore DDR heterogeneity and adaptability. Profiling of patient-derived lines reveals recurrent driver mutations and DDR-enriched transcriptional clusters with prognostic relevance[88]. Combined Nbs1/p53 loss generates highly unstable gliomas resembling pediatric and post-radiotherapy tumors[89]. Three-dimensional platforms further show that Tumor Treating Fields synergize with TMZ and PARP inhibitors independently of p53 or *MGMT* status, reducing BRCA1 levels and impairing DNA repair[90,91]. In parallel, ALT glioma models further support tolerance of persistent, non-lethal genome maintenance states[92].

Together, these studies establish DDR plasticity as a central, multi-layered survival program in GSCs, in which signaling, microenvironmental cues, and stress-adaptive responses dynamically rewire DDR outputs to favor persistence under genotoxic stress.

Direct reversal and base excision repair in chemoradioresistance

Downstream of checkpoint activation and replication-stress tolerance, GSCs rely on direct reversal and base excision repair (BER) against alkylation-induced DNA damage. *MGMT*-mediated reversal of O6-methylguanine lesions and PARP-dependent BER critically buffer the cytotoxic effects of TMZ and radiotherapy, sustaining clonogenic survival under genotoxic stress.

PARP1 inhibition disrupts BER and converts repairable lesions into replication-associated DSBs in an MGMT-dependent manner[93]. MGMT itself remains a central determinant of therapy response: type I interferons suppress *MGMT* transcription via NF-κB inhibition[94], PRIMA-1^{MET} reduces *MGMT* expression and stemness independently of p53[95], while hypoxia enhances both MGMT levels and stem-like traits[96]. Consistently, MEK-ERK signaling likewise sustains *MGMT* expression and temozolomide resistance through the MDM2-p53 axis, linking *MGMT* regulation to oncogenic signaling[97]. Conversely, delphinidin glycosides inhibit NF-κB-driven *MGMT* transcription and synergize with TMZ, particularly in mesenchymal GSC populations[98]. Therapeutic MGMT blockade can also be achieved through targeted delivery strategies, as Biomimetic co-delivery of TMZ with an MGMT inhibitor suppresses orthotopic glioblastoma growth, supporting direct reversal as a tractable vulnerability in vivo[99]. MGMT targeting also radiosensitizes GSCs by prolonging γH2AX persistence and promoting mitotic catastrophe after irradiation[100]. Nanoparticle-mediated silencing of drug-resistance programs likewise prolongs survival in orthotopic glioblastoma models[101].

Replication-associated buffering mechanisms functionally intersect with direct reversal and BER. Telomestatin selectively induces telomeric dysfunction and ATR-Chk1 hyperactivation in GSCs, triggering replication-dependent arrest and death[48]. Resolution of replication-associated topological stress depends on topoisomerase IIβ, whose depletion sensitizes GSCs to TMZ and alkylating agents[49], whereas RPA inhibition causes extensive DSB accumulation and radiosensitization[50]. Consistently, PARP inhibition with talazoparib markedly enhances radiosensitivity, particularly with high-LET carbon ions, by preventing DSB resolution and enforcing durable G2/M arrest[102]. In this context, ATR-Chk1 signaling becomes a critical compensatory dependency, and ATR inhibition synergizes with TMZ specifically in *MGMT*-methylated GSCs[74]. BER-centered defenses are further modulated by hypoxia, as the lncRNA *LUCAT1* regulates DDR output in hypoxic GSCs[103].

Cell state and microenvironment further modulate these repair-centric defenses. Dedifferentiation driven by *RB* loss, *KRAS* activation, and *PTEN* inactivation generates a stem-like, *MGMT*-positive TMZ-resistant phenotype[104], while tumors arising near the subventricular zone exhibit enriched stemness programs, elevated MGMT signaling, and inferior outcome[105]. Therapeutic strategies increasingly exploit these vulnerabilities: dual ALKBH2/ALKBH5 inhibition reverses TMZ resistance and reduces MGMT expression[106]. Tumor Treating Fields sensitize GSCs to PARP inhibition and TMZ independently of p53 and MGMT status[91], *PDCD10* loss promotes MGMT upregulation and TMZ tolerance[107], and differentiation therapy with all-trans retinoic acid downregulates MGMT and stemness, restoring TMZ sensitivity[108]. Oltipraz also shows antitumor activity in glioblastoma, although its connection to MGMT or BER appears indirect[109].

Together, these studies indicate that direct reversal and BER operate within a broader replication-stress-adaptive network in which MGMT regulation, PARP activity, and fork protection sustain therapy resistance. Targeting these interconnected layers may therefore dismantle repair-centered defenses in HGG, positioning BER and direct reversal as proximal buffering layers within a broader adaptive DDR network.

Homologous recombination dynamics

Following checkpoint addiction and replication-stress tolerance, HR emerges as a key determinant of therapy response heterogeneity. In GSCs, HR spans a continuum from defective, genomically unstable states to RAD51-dependent HR-addicted phenotypes.

Early evidence indicates that not all GSCs are radioresistant: CD133^{+} populations can exhibit impaired HR and incomplete checkpoint activation, leading to radiosensitivity and chromosomal instability[110]. However, subsequent studies predominantly identified reinforced HR states. RAD51 is consistently upregulated in radioresistant GSCs and further induced by irradiation; its pharmacologic inhibition (RI-1, B02) suppresses RAD51 foci, disrupts HR, induces persistent DSBs and apoptosis, and selectively collapses clonogenic survival while sparing normal neural stem cells[111,112]. Clinically, high *RAD51* expression correlates with poor response and shorter progression-free survival.

This HR dependency is sustained by convergent transcriptional and regulatory circuits. STAT3-dependent FOXM1 signaling transcriptionally controls core HR genes, and its inhibition increases γH2AX, impairs HR, and induces mitotic catastrophe[113]. The lncRNA *DARS1-AS1* stabilizes *FOXM1*, *RAD51*, and *BRCA1* transcripts via YBX1, sustaining HR and tumor growth; its depletion radiosensitizes GSCs in vivo[114]. *BRCA1* itself modulates TMZ response in *p53* wild-type GSCs, where its knockdown suppresses HR activation and increases apoptosis[115]. PRMT5 inhibition likewise impairs Fanconi anemia pathway-mediated HR and enhances temozolomide efficacy, supporting FA/HR-associated repair dependencies as actionable vulnerabilities[116]. Likewise, MEOX2 promotes DNA repair and therapy resistance in glioblastoma stem-like cells through PARP1 interaction, indicating that HR competence depends on broader regulatory networks beyond RAD51 abundance alone[117].

HR addiction is therapeutically exploitable. Artesunate synergizes with TMZ by suppressing RAD51 and HR without increasing primary DSB burden[118]. Oncolytic HSV combined with PARP inhibition induces proteasomal degradation of RAD51 and CHK1, causing massive DSB accumulation and apoptosis selectively in GSCs; RAD51 knockdown phenocopies PARPi sensitivity, confirming HR disruption as the synthetic-lethal driver[119]. Conversely, resistance to PARP inhibition can arise through a Myc-CDK18-ATR axis: *Myc*-amplified cells exhibit an HR-defective, PARPi-sensitive state, while CDK18-driven ATR activation restores HR in non-amplified cells; inhibition of CDK18 or ATR re-sensitizes these populations[120]. In line with this, lipid metabolic regulation also feeds into HR-associated repair, as SCD1 and SCD5 modulate PARP-dependent DNA repair through fatty acid desaturation[121].

HR regulation further integrates chromatin and stress-adaptive pathways. The elongation factor SPT6 maintains *BRCA1* and *RAD51* expression; its inhibition suppresses HR, induces G2 arrest, polyploidy, and loss of tumorigenicity[122]. RECQL4 depletion disrupts HR, activates checkpoint signaling, and sensitizes GSCs to TMZ[123]. Telomere-directed stress also intersects with this circuitry, as G-quadruplex stabilization imposes replication-associated stress incompatible with GSC survival[124].

Metabolic and ER-stress signaling converge on HR through SCD1, which sustains *RAD51* expression downstream of IRE1-SREBP1; its inhibition induces persistent DNA damage and near-complete GSCs depletion[125]. In 3D cultures, VEGF-Akt signaling promotes HR and DNA-PKcs activation, whereas its inhibition causes durable γH2AX accumulation and radiosensitization[126]. The chaperone HSP90 stabilizes RAD51 and CHK1; onalespib-mediated degradation of both proteins abrogates HR and enhances radio- and chemosensitivity[127].

Finally, post-translational and epitranscriptomic regulators reinforce HR proficiency. The UCHL3-POLD4 axis stabilizes replication polymerase POLD4, sustaining HR/NHEJ and radioresistance[128], whereas the MST4-USP14-ALKBH5 module preserves *RAD51* expression via m6A-dependent stabilization; its disruption impairs HR and radiosensitizes GSCs[129]. In vivo genetic modeling using RCAS-TVA-CRISPR systems further enables functional dissection of HR dependencies and synthetic-lethal vulnerabilities during glioma evolution[130].

Together, these studies define HR as a dynamically regulated vulnerability ranging from HR-deficient to HR-addicted states. Across this spectrum, RAD51-centered HR networks, integrated with transcriptional, metabolic, and stress-response pathways, represent a central and targetable axis of therapy resistance in HGG.

DNA-PK-dependent end joining

Whereas homologous recombination defines a major resistance axis in proliferating GSCs, non-homologous end joining (NHEJ) provides a complementary and often dominant survival strategy under replication stress, quiescence, and acute irradiation. In GSCs, rapid, cell-cycle-independent DSB repair mediated by DNA-PKcs and XRCC4 enables

survival after genotoxic stress at the cost of increased mutagenic tolerance.

Multiple studies demonstrate a selective reliance of GSCs on DNA-PK-driven NHEJ. Depletion of DNA-PKcs impairs DSB repair and redirects damaged cells toward LC3-Beclin-1-dependent autophagic cell death through mTOR inhibition, mechanistically linking defective NHEJ to autophagic vulnerability[131]. Consistently, temporal DNA-PK activation has been shown to drive genomic instability and therapy resistance in GSCs, supporting the view that persistent NHEJ signaling promotes survival at the cost of genome integrity[132]. Cathepsin L depletion likewise enhances radiosensitivity in vitro and in vivo, further supporting NHEJ-linked stress tolerance as a therapeutically actionable dependency[133]. Consistently, NHEJ dominance over apoptotic signaling and persistence in quiescence are defining features of GSCs exposed to genotoxic stress[134].

This dependency is tightly modulated by oncogenic signaling. Akt inhibition robustly radiosensitizes GBM cells, particularly those harboring mutant or lost *TP53*, by reducing DNA-PKcs levels and crippling NHEJ-mediated DSB repair. Silencing *TP53* in wild-type cells phenocopies this vulnerability, indicating that *TP53* loss enforces compensatory reliance on PI3K-Akt-NHEJ signaling. Combined Akt and DNA-PKcs inhibition radiosensitizes even p53-proficient cells, supporting cooperative targeting of this axis[135]. Direct pharmacologic exploitation of this circuitry is exemplified by the EGFR-directed combi-molecule ZR2002, which suppresses EGFR/Erk/Akt signaling while inducing DNA damage and selectively eliminates TMZ-resistant, EGFRvIII-positive GSCs in vivo in a p53-dependent manner[136]. This concept is reinforced by ZYH005, which similarly couples EGFR targeting to DNA damage induction and mitotic catastrophe in glioblastoma[137].

Spatial context further shapes NHEJ reliance. In 3D GSC models, VEGF-Akt signaling activates DNA-PKcs and sustains both NHEJ and HR; VEGF deprivation abrogates these pathways, induces persistent γH2AX and pDNA-PKcs foci, and triggers mitotic catastrophe with profound radiosensitization. Akt inhibition reproduces these effects across both 2D and 3D systems, improving survival in vivo[126].

Post-translational regulation integrates NHEJ with stemness and plasticity. In mesenchymal GSCs, the UCHL3-POLD4 axis stabilizes polymerase δ, supporting efficient NHEJ and HR, self-renewal, and tumorigenicity; its disruption induces persistent DSBs, loss of stemness, and potent radiosensitization[128]. Transcription-associated repair scaffolds further reinforce NHEJ function. AATF, upregulated in GSCs, binds and stabilizes XRCC4 at replication forks; following DNA damage, ATM-dependent phosphorylation releases XRCC4 to enable DSB repair. Disrupting this interaction impairs NHEJ, increases unrepaired damage and apoptosis, and sensitizes tumors to radio- and chemotherapy[138].

Together, these studies establish DNA-PKcs- and XRCC4-dependent NHEJ as a dominant, druggable survival pathway in GSCs that cooperates with oncogenic signaling and niche cues to sustain DSB repair and stress tolerance.

Beyond canonical DNA repair pathways, GSC resistance is further sustained by regulatory layers that do not directly remove DNA lesions, but instead maintain stemness, stress tolerance, and the broader cellular conditions required for adaptive DDR states. The following sections focus on signaling, chromatin and RNA-based regulation, translation, and metabolism as modules shaping DDR adaptation in GSCs.

PI3K/Akt signaling as an upstream regulator of stemness and DDR adaptation

The PI3K/Akt pathway is one of the most consistently activated survival programs in GSCs and acts primarily as an upstream regulator of stemness-associated survival rather than as a direct DNA repair pathway. By integrating mitogenic, metabolic, and stress-adaptive signals, Akt establishes a permissive context in which GSCs suppress apoptosis and sustain recovery after genotoxic stress. PI3K/Akt signaling, therefore, acts as a coupling layer between stemness and DDR adaptation. This view is further supported by studies showing that pharmacologic inhibition of VEGF/PI3K/Akt signaling or dual PI3K/mTOR blockade is sufficient to induce cell-cycle arrest, apoptosis, and radiosensitization in GSCs, consistent with a role in maintaining adaptive survival states rather than directly executing DNA repair[139,140].

Notch signaling provides a prototypical example of Akt-dependent radioresistance. Following irradiation, Notch activation promotes GSC survival by engaging PI3K/Akt and modulating anti- versus pro-apoptotic Mcl-1 isoforms, without increasing intrinsic DNA repair capacity. Pharmacologic inhibition of Notch using γ-secretase inhibitors selectively radiosensitizes the $CD133^{+}$ stem-like fraction while sparing normal neural cells, highlighting a GSC-specific dependence on this axis[141]. GSCs also sustain Akt activation through a distinct autocrine sphingosine-1-phosphate (S1P) loop. Unlike normal neural cells, GSCs constitutively produce and secrete S1P, activating S1P1-Akt signaling to maintain survival under genotoxic stress. This signaling limits DSB accumulation and supports viability even when MGMT-mediated repair is compromised, identifying S1P as a critical upstream regulator of Akt-driven resistance[142].

Disruption of PI3K/Akt signaling unmasks pronounced vulnerabilities. Hyperthermia prevents radiation-induced Akt activation, abolishes self-renewal, induces persistent DSBs and apoptosis, and suppresses tumor growth. In vivo, combined hyperthermia-radiotherapy significantly prolongs survival, with Akt inhibition driving radiosensitization[143]. Recent work further extends this concept upstream and downstream of Akt signaling: *PTEN* reactivation impairs GSCs by disrupting cytosolic iron-sulfur assembly, whereas brain-targeted co-delivery of osimertinib and bortezomib suppresses radioresistant glioblastoma in a differentiation-informed therapeutic setting, supporting the tractability of PI3K/Akt-regulated resistant states[144,145].

Together, these studies indicate that PI3K/Akt signaling does not primarily enhance DNA repair, but preserves stemness-associated survival states in which adaptive DDR programs can be deployed. Targeting this pathway may therefore be required to dismantle the adaptive survival state of tumor-maintaining GSCs.

Epigenetic and epitranscriptomic control of stemness and DDR competence

Epigenetic and epitranscriptomic regulation are central determinants of DDR competence and therapy resistance in GSCs. By coordinating the control of chromatin structure, histone modifications, DNA/RNA methylation, and protein stability, GSCs establish a plastic regulatory landscape that reinforces DNA repair and adaptive survival under genotoxic stress. Chromatin- and RNA-based mechanisms, therefore, represent complementary layers of DDR regulation. Disrupting these circuits may therefore destabilize adaptive DDR states.

RNA methylation has emerged as a major regulatory layer. METTL3-dependent m6A methylation is highly enriched in GSCs and stabilizes *SOX2* mRNA via 3′UTR modification, sustaining stemness and radioresistance; METTL3 depletion reduces neurosphere formation, increases γH2AX persistence, and radiosensitizes GSCs, effects rescued by *SOX2* lacking the regulated 3′UTR[146]. METTL3 also promotes chemoresistance by stabilizing *MGMT* and *APNG* transcripts, lowering TMZ sensitivity in vitro and in vivo when depleted[147].

Global DNA and RNA methylation patterns further distinguish GSCs from normal neural stem cells. GSCs exhibit reduced 5mC/5hmC, increased 5fC/5caC, aberrant TET activity, and enhancer remodeling linked to proliferation and therapy tolerance. TET2 overexpression supports stemness maintenance and DNA repair efficiency, implicating TET-dependent oxidation programs in survival under therapy[148].

m6A demethylases provide an additional layer of DDR control. The m6A demethylase FTO promotes radioresistance and stemness maintenance in GSCs, supporting reversible RNA methylation as a regulator of DDR adaptation[149]. Likewise, ALKBH5, highly expressed in GSCs, promotes radioresistance and invasion by sustaining HR proficiency through regulation of *CHEK1*, *RAD51*, and other HR genes; its inhibition radiosensitizes GSCs and suppresses growth in 3D models[150]. At the post-

translational level, the MST4-USP14-ALKBH5 axis prevents ubiquitin-mediated ALKBH5 degradation, preserving *RAD51* expression and HR capacity; disruption of this pathway induces persistent DNA damage, apoptosis, and radiosensitization[129].

Histone-modifying enzymes also critically shape DDR outcomes. The lysine demethylase KDM1A/LSD1, overexpressed in GSCs, transcriptionally activates HR and NHEJ genes, including *BRCA1*, *RAD51*, and *FOXM1*; its inhibition suppresses DSB repair, increases γH2AX accumulation, reduces stemness, and sensitizes GSCs to TMZ in vivo[151]. Similarly, KDM2B supports glioma stem-like cell survival and chemoresistance, extending the role of histone demethylases beyond LSD1 to a broader epigenetic framework that preserves resistant stem-like states[152]. Similarly, G9a/GLP inhibition sensitizes both glioma cells and GSC-enriched neurospheres to TMZ by inducing apoptosis and autophagy, independently of *MGMT* promoter methylation or stemness gene expression, identifying G9a as a broad epigenetic vulnerability across GBM cell states[153]. HDAC6 inhibition promotes GSC differentiation and radiosensitivity through SHH/Gli1 suppression, further linking stemness maintenance to DDR competence[154].

Clinically relevant signals further intersect with epigenetic DDR regulation. Dexamethasone induces a CEBPB-driven transcriptional program that enhances proliferation, survival, and TMZ resistance, with upregulation of DNA repair genes such as *XRCC2* and *BRCA2*. This mesenchymal repair-competent state can be antagonized by camptothecin, suggesting that steroid exposure may reinforce DDR-proficient states[155]. Ionizing radiation itself can also reshape this layer by regulating *MYC* and *NBN* expression, suggesting that therapy-induced transcriptional responses feed back into epigenetically controlled DNA repair programs[156].

Epigenetic reactivation of tumor suppressors also modulates therapy response. The DNA methylation inhibitor 5-azacitidine restores the expression of *TUSC3*, reducing stemness and re-sensitizing GSCs to TMZ independently of *MGMT* status; in *MGMT*-unmethylated lines, combination with lomeguatrib enables robust tumor suppression[157].

Finally, higher-order chromatin organization directly couples inflammatory signaling to DDR. NUP98, acting as a chromatin scaffold through interaction with NF-κB (p65/RelA), sustains transcription of core HR factors including *BRCA1/2*, *RAD51*, *RAD54L*, *BLM*, and *XRCC2*. NUP98 depletion collapses HR capacity, increases post-irradiation apoptosis, reduces stemness, and sensitizes tumors to radio-chemotherapy, identifying NUP98-NF-κB as a chromatin-based DDR hub[158]. At the same time, miR-128-mediated targeting of Polycomb repressor complexes further indicates that non-coding RNA networks epigenetically restrain stem-like programs[159].

Together, these studies establish epigenetic and epitranscriptomic regulation as a major layer coordinating DDR competence in GSCs. Targeting RNA methylation, histone modifiers, chromatin scaffolds, and protein-stability circuits may therefore destabilize treatment-refractory GSC states.

RNA splicing as a post-transcriptional link between stemness and DDR competence

Building on epigenetic regulation, RNA splicing emerges as a post-transcriptional layer through which GSCs fine-tune stemness and DDR competence. Alterations in spliceosome integrity and alternative splicing directly shape DNA repair and oncogenic signaling networks.

The spliceosomal component SNRPB is overexpressed in GBM and patient-derived GSCs, with high levels correlating with poor prognosis. *SNRPB* knockdown induces apoptosis and widespread splicing disruption, including intron retention and downregulation of genes involved in RNA processing, chromatin remodeling, HR, and DNA repair. Affected pathways include RTK, PI3K-AKT, RAS/MAPK, RB, and p53, indicating that spliceosome integrity is required to maintain DDR-proficient stem-like programs[160]. Splicing regulation also underlies functional heterogeneity across GSC subtypes. Comparative analyses of proneural and mesenchymal GSCs identify ~4900 differential splicing events, with strong concordance between in vitro models and patient tumors. Mesenchymal GSCs preferentially exhibit alternative splicing of DNA repair and cell-cycle genes (*ERCC1*, *FANCD2*, *RAD17*) and increased expression of prognostic lncRNAs (*CRNDE*, *MYOSLID*, *SOX21-AS1*), correlating with enhanced radioresistance[161]. More broadly, post-transcriptional RNA regulation beyond core spliceosome function also contributes to resistant stem-like states, as miR-146b-5p and proneurogenic miRNA programs modulate stemness and therapy response in GSCs[162,163].

Together, these findings indicate that aberrant RNA splicing contributes directly to the acquisition and maintenance of DDR competence in stem-like glioma states. This supports spliceosome-associated regulators as therapeutic vulnerabilities in HGG.

Translational control of DDR competence

Beyond checkpoint activation and repair pathway selection, GSCs depend heavily on translational control to sustain DDR signaling after genotoxic stress. Following irradiation, rapid replenishment of short-lived repair and checkpoint proteins becomes rate-limiting, rendering protein synthesis a key determinant of survival. Recent translatome-level analyses further support a major role for mRNA translation in adaptive glioblastoma biology[164].

The mTOR pathway is central to this dependency. Dual mTORC1/2 inhibition with AZD2014 (Vistusertib) impairs post-irradiation recovery, causing persistent γH2AX and delayed DSB resolution by suppressing eIF4E/4E-BP1-dependent cap-dependent translation of DDR proteins[165]. Polysome profiling confirms that radiation responses in GSCs are predominantly regulated at the translational level: INK128 disrupts eIF4F assembly, prolongs DNA damage signaling, alters organelle remodeling, and markedly radiosensitizes GSCs[166].

Metabolic inputs converge on this axis. G0S2, upregulated in radioresistant GSCs and recurrent GBM, activates mTOR-S6K signaling and stabilizes 53BP1 through RNF168 suppression, promoting efficient DSB repair. Targeting G0S2 disrupts translational signaling, increases DNA damage, and restores radiosensitivity[167].

Translational dependence is further reinforced by control of ribosome biogenesis. Inhibition of XPO1 with selinexor blocks nuclear export of 5S and 18S rRNA, suppresses polysome formation, and prevents synthesis of essential DDR proteins. Combined with irradiation, selinexor induces persistent γH2AX, unrepaired DSBs, and significantly prolongs survival in orthotopic models, while sparing normal neural tissue[168]. In parallel, extrinsic ribosome stimuli can drive glioma cells toward stem-like states, supporting a role for ribosome-linked translational control in maintaining DDR-competent stem-like states[169].

Together, these studies place translational control downstream of stemness-associated survival circuitry and upstream of repair execution, identifying it as a systems-level determinant of adaptive DDR competence. This exposes a broader vulnerability that can be exploited to overcome radioresistance in GSCs.

Metabolic reprogramming and DDR support

Effective DDR execution in GSCs is tightly coupled to metabolic fitness. Metabolic rewiring provides the energetic, redox, and biosynthetic conditions required for adaptive DDR states under therapy.

Early studies showed that resistant GSCs adopt a caloric restriction-like metabolic state characterized by reduced glycolysis, enhanced β-oxidation, and constitutive AMPK/SIRT1-PGC-1α activation promoting autophagy and DDR reinforcement[170]. Disrupting this state sensitizes GSCs: resveratrol plus irradiation suppresses clonogenicity and impairs DSB repair in vivo[171], whereas ATP depletion by D609 triggers GSC death independently of direct DNA damage[172]. Consistently, compromising mitochondrial fitness with 3-acetonyltabersonine selectively kills GSCs by inducing mitochondrial depolarization, suppressing ATM signaling, and impairing post-damage recovery[173]. CRISPRi screens further link metabolic stress responses to chemoresistance programs in glioblastoma[174].

Metabolic-DDR coupling also emerges through oxidative stress and lipid signaling. The compound NEO100 induces ER stress and apoptosis while suppressing invasion and prolonging survival[175]. *EGFR*-amplified GSCs, characterized by chronic ROS and elevated baseline DDR, show a selective dependence on PARP1 and exquisite sensitivity to talazoparib[176]. At the population level, NRF2 maintains antioxidant defenses and stemness; its depletion increases oxidative stress and radiosensitizes GSCs to photons and carbon ions[177]. Chaperone-mediated autophagy further intersects with metabolic regulation by promoting degradation of enzymes such as IDH1, linking autophagy to metabolic-DDR coupling[178].

Direct metabolic control of DNA repair has recently been uncovered. ALDH1A3 drives glycolytic flux and lactate-dependent XRCC1 lactylation, promoting BER/NHEJ under TMZ and radiation; disrupting the ALDH1A3-PKM2 interaction restores chemosensitivity[179]. Similarly, PHGDH-dependent serine synthesis sustains one-carbon metabolism, nucleotide supply, and HR proficiency; PHGDH inhibition increases ROS and DSBs and synergizes with radiation in PDX models[180]. The PGK1/PHGDH axis likewise drives radioresistance in GSCs, reinforcing metabolic support of DDR competence[181]. Lipid metabolism also directly modulates repair capacity: de novo lipid synthesis supports DNA repair, whereas its inhibition impairs DDR, and lipid droplet formation counteracts PARP inhibition[182,183].

Microenvironmental and treatment-related factors further shape metabolic DDR states. Periventricular GSC niches exhibit enhanced stemness and DNA repair signaling[184], while MRSI-defined GSC-rich regions correlate with aggressive metabolic and DDR programs[185]. Ex vivo, neurosphere cultures show greater radioresistance and slower γH2AX resolution than tumor bulk[186]. Macrophage-derived lactate suppresses cGAS-STING signaling and antitumor immunity, whereas lactate transport inhibition restores immune activation, linking lactate metabolism to DDR signaling and immune evasion[187]. Nuclear cholesterol also contributes to DDR regulation by controlling nuclear architecture and DNA damage responses in cancer stem cells[188]. High-LET carbon ions induce complex, slowly repaired damage in GSCs[189], whereas fractionated radiotherapy can enrich $CD133^{+}$ GSCs and promote mitochondrial biogenesis and invasiveness[190]. Therapeutically, IKCa/BKCa channel inhibition radiosensitizes patient-derived GSCs[191], and an oncolytic HSV-1 armed with a BiTE exploits DNA damage-induced NKG2DL expression to amplify immune-mediated killing[192]. Iron metabolism also contributes to radiation-response heterogeneity, as ferritin heavy chain modulation alters sensitivity in glioblastoma-initiating cells[193].

Together, these studies indicate that metabolism functions as a permissive layer of adaptive DDR competence, enabling stem-like glioma cells to buffer genotoxic stress and preserve long-term tumor-propagating capacity. Targeting these adaptive metabolic layers, therefore, offers a powerful route to destabilize therapy-resistant GSC states and enhance chemoradiotherapy efficacy.

Therapeutic implications of targeting DDR plasticity in GSCs

The multilayered DDR architecture described above reveals several tractable therapeutic vulnerabilities in GSCs.

Clinically, *MGMT* promoter methylation remains the strongest predictive biomarker for temozolomide response in *IDH*-wildtype glioblastoma, where it predicts benefit from alkylating chemotherapy in randomized trials[194,195]. In contrast, its predictive utility is limited in *IDH*-mutant gliomas, where *MGMT* methylation is nearly universal and does not reliably predict temozolomide benefit[196,197]. Beyond MGMT, replication stress and ATR–Chk1 dependency provide a rationale for combining ATR inhibitors, such as ceralasertib or gartisertib, with radiotherapy or temozolomide, particularly in *MGMT*-methylated tumors, although brain penetrance remains limiting for some agents such as berzosertib[198–200]. PARP inhibition, including olaparib and talazoparib, shows genotype-selective activity in *EGFR*-amplified GSCs, where EGFR-induced oxidative stress creates dependence on PARP-mediated BER, and in tumors with *MYC/MYCN* amplification or *IDH* mutations that confer a "BRCAness" phenotype amenable to synthetic lethality[120,176,201,202]. RAD51- and DNA-PKcs-targeting approaches have also entered clinical evaluation; for example, peposertib, a DNA-PKcs inhibitor, is being evaluated clinically in newly diagnosed *MGMT*-unmethylated glioblastoma (NCT04555577), with favorable safety and median OS of 22.9 months[203]. However, radiosensitization by DDR inhibitors may affect tumor and normal tissues in parallel, making therapeutic window optimization a central issue[204,205]. Additional opportunities emerge from epigenetic and epitranscriptomic targeting. METTL3 inhibition is particularly attractive because m6A-dependent regulation supports HR repair, stabilizes *SOX2*, and enhances *MGMT/APNG* expression, thereby promoting TMZ resistance[146,206–209]. LSD1 inhibition primarily disrupts GSC maintenance through deregulation of the ATF4-dependent integrated stress response, inducing differentiation and senescence rather than directly suppressing DDR genes[210], whereas G9a inhibition can impair both HR and NHEJ repair and radiosensitizes glioma cells, although effects on stemness appear context-dependent[211–215]. Metabolic dependencies including PHGDH, SCD1, and ALDH1A3 are also being evaluated in orthotopic models, although GBM-specific clinical trials remain limited[179,180,216–218].

Finally, translational control through mTOR and XPO1 inhibition is clinically advanced: selinexor has completed phase II trials in recurrent GBM (PFS6 17%) and is being evaluated in combination with standard therapy (NCT04421378), although it may paradoxically induce *MGMT* expression in *MGMT*-unmethylated tumors and antagonize TMZ efficacy[168,219–222].

Effective therapy will likely require combination regimens simultaneously targeting checkpoint addiction (ATR/Chk1), repair execution (RAD51/DNA-PK), and adaptive survival programs (PI3K/Akt, epigenetic plasticity), guided by molecular DDR and metabolic biomarkers rather than histology alone. The main targetable axes, representative compounds, and candidate selection markers are summarized in Table 3.

Discussion and conclusion

The literature reviewed here supports a framework in which therapy resistance in HGGs is sustained by a multilayered and plastic DNA damage response. Reinforced checkpoint signaling, replication-stress tolerance, dynamic DNA repair, epigenetic regulation, translational adaptation, and metabolic support collectively enable GSC survival and recurrence. Within this framework, preservation and recovery of transcriptional activity after DNA damage emerge as additional dimensions of genome maintenance.

This perspective is particularly relevant because GSCs display elevated transcriptional output and preferential expression of long neural genes associated with sustained RNA polymerase II occupancy[223,224]. Long genes are prone to transcription-replication conflicts, R-loop accumulation, and endogenous replication stress, contributing to constitutive DDR activation[225,226]. In GSCs, chronic replication stress driven by transcription of long neural genes has been directly linked to persistent ATR-Chk1 signaling and radioresistance[45]. In this transcription-intensive state, transcriptional competence is tightly coupled to stemness and stress adaptation. Consistent with this view, mechanistic studies on highly transcribed genomic domains have shown that transcriptional activity itself shapes repair prioritization. Work on ribosomal DNA has shown that transcription-coupled repair preferentially preserves transcription at highly active loci, supporting a hierarchy in genome maintenance that favors transcription recovery over global lesion removal[227]. These findings suggest that cells under high transcriptional demand actively protect transcriptionally critical regions following DNA damage. Recent glioma studies further show that ionizing radiation induces rapid P-TEFb-dependent reorganization of RNA polymerase II occupancy and elongation programs, supporting transcriptional recovery as an active adaptive response contributing to post-irradiation survival[228].

From a chemical standpoint, DNA damage induced by standard therapies is heterogeneous and generates lesions with distinct structural consequences. Temozolomide predominantly forms N7-methylguanine and N3-methyladenine adducts, along with abasic sites. These lesions are weakly helix-distorting and are therefore classically assigned to BER. However, alkylated bases, abasic sites, and oxidized nucleotides can

https://doi.org/10.1038/s42003-026-10467-7

Table 3 | Therapeutic playbook for targeting DDR plasticity in glioma stem cells

Target Axis	Representative Compounds / Clinical Status	Brain Penetrance	Rationale for Combination (TMZ/RT/TTFields)	Potential Patient Selection Markers
ATR/CHK1	M6620 (Berzosertib), AZD6738 (Ceralasertib)	Limited – berzosertib Moderate - ceralasertib	Abrogates G2/M checkpoint; synergizes with TMZ in *MGMT*-meth GSCs by inducing replication fork collapse	*MGMT* promoter methylation; High replication stress markers
RAD51/HR	RI-1, B02, CYT-0851	Under evaluation	Prevents repair of RT-induced DSBs; overcomes "HR-addiction" in GSCs after genotoxic stress.	High *RAD51* expression; HR-dependency signatures.
DNA-PKcs / NHEJ	M3814 (Nedisertib), AZD7648	Moderate/under evaluation	Potent radio-sensitizer; blocks the primary repair pathway for RT-induced DSBs in GSCs.	DNA-PKcs overexpression; Low HR activity.
PI3K–AKT	Paxalisib (GDC-0084), Buparlisib	High	Inhibits non-canonical DDR signaling and metabolic survival pathways post-irradiation.	PTEN loss; PIK3CA mutations; AKT phosphorylation.
MET	Capmatinib, Tepotinib	Moderate	Blocks MET-induced radioresistance and pro-survival signaling in the perivascular niche.	MET amplification or high HGF expression.
Notch	CB-103, LY3039478	Moderate	Targets the GSC quiescent pool; inhibits Notch-mediated upregulation of DDR genes.	Notch intracellular domain (NICD) levels.
LSD1	GSK2879552, Seclidemstat	Under evaluation	Epigenetic silencing of DDR genes (*BRCA1*/*RAD51*); forces GSC differentiation making them sensitive to RT.	GSC-specific transcriptional signatures.
ALKBH5 / METTL3	Ena15, Experimental inhibitors	Under development	Modulates m6A-dependent stability of HR mRNAs; enhances RT sensitivity.	High *ALKBH5* expression; m6A RNA methylation levels.
SCD1 / PHGDH	A939572 (SCD1), NCT-503 (PHGDH)	Limited (experimental)	Targets metabolic vulnerabilities (lipid/serine metabolism) required for membrane and DNA synthesis during repair.	High lipid droplets; *PHGDH* overexpression.
XPO1 / mTOR	Selinexor (XPO1), Vistusertib (mTOR)	High (Selinexor)	Impairs nuclear export of DDR proteins (XPO1) and protein synthesis required for repair (mTOR).	XPO1 nuclear localization; mTORC1/2 activation.

This table summarizes the main targetable DDR-related axes discussed in the present Review, together with representative compounds, available evidence on brain penetrance, rationale for combination with radiotherapy, temozolomide, or other genotoxic strategies, and candidate biomarkers that may help identify tumors more likely to depend on specific adaptive DDR states. Rather than providing an exhaustive clinical overview, the table is intended to highlight translationally relevant vulnerabilities emerging from the multilayered DDR architecture of GSCs. *TMZ* temozolomide, *RT* radiotherapy, *TTFields* Tumor Treating Fields, *GSCs* glioma stem cells, *DDR* DNA damage response, *DSBs* double-strand breaks, *HR* homologous recombination, *NHEJ* non-homologous end joining, *RT-induced* radiotherapy-induced, *HGF* hepatocyte growth factor, *NICD* Notch intracellular domain, *m6A* N6-methyladenosine.

efficiently block elongating RNA polymerase II on transcribed strands independently of lesion bulk[229]. Accordingly, transcription-coupled repair is functionally defined by transcriptional arrest rather than by lesion chemistry per se[230,231].

Ionizing radiation further reinforces this rationale. Beyond DSBs, radiation generates oxidized bases, clustered lesions, and helix distortions[232,233]. Many of these lesions interfere with transcriptional elongation even after DSB repair. Recovery of transcription after ionizing radiation is an active process genetically separable from DSB repair capacity. Cells proficient in HR and NHEJ but impaired in transcription-coupled repair exhibit persistent transcriptional arrest and radiosensitivity, indicating that transcription recovery represents a distinct DDR outcome[234–236]. Recent evidence further identifies PHF8 as a facilitator of transcription recovery after DSB repair, supporting restoration of RNA polymerase II activity as a regulated post-damage process[237].

These principles are particularly relevant in glioma stem cells. In GSCs, where continuous transcription sustains stemness and adaptive signaling, restoration of transcription after DNA damage may represent a critical priority. This view is consistent with observations that GSC resistance relies on damage tolerance and prolonged checkpoint engagement rather than complete lesion removal[37,41,45].

Although replication stress and transcriptional plasticity are established features of GSC biology, the contribution of transcription-associated repair and transcription restart to therapy response remains incompletely understood. Recent work has begun to define the molecular choreography underlying transcription restart in response to transcription-blocking lesions, identifying regulators such as XAB2 that coordinate RNA polymerase II release and chromatin remodeling during transcription restart[238,239]. Future studies should determine whether transcriptional recovery contributes functionally to GSC therapy response using RNA polymerase II occupancy profiling, nascent RNA labeling, and dynamic analysis of XAB2 following irradiation[228,238,240]. It will also be important to determine whether transcriptional recovery is mechanistically linked to HR programs and translation-dependent repair-factor availability. In this regard, *SPT6*-dependent maintenance of *BRCA1* and *RAD51* expression together with mTOR-dependent translation of repair factors suggests that transcription restart may be embedded within HR- and translation-linked survival circuits[122,165,166].

Taken together, these considerations suggest that integrating transcription-associated repair and transcription recovery into current DDR frameworks may provide a more comprehensive view of how glioma stem cells withstand therapy-induced damage. While checkpoint addiction, replication-stress management, HR, NHEJ, epigenetic regulation, translational control, and metabolic rewiring remain central resistance mechanisms, transcriptional recovery may represent an additional functional endpoint of genome maintenance relevant to recurrence in HGGs.

Received: 9 February 2026; Accepted: 2 June 2026;
Published online: 09 June 2026

References

1. Stupp, R. et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol.* **10**, 459–466 (2009).
2. Morello, A. et al. Laser interstitial thermotherapy (LITT) in recurrent glioblastoma: what window of opportunity for this treatment? *Technol. Cancer Res. Treat.* **23**, 15330338241249026 (2024).
3. Stupp, R. et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* **352**, 987–996 (2005).
4. Kreisl, T. N. et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. *J. Clin. Oncol.* **27**, 740–745 (2009).
5. Louis, D. N. et al. The 2021 WHO classification of tumors of the central nervous system: a summary. *Neuro-Oncol.* **23**, 1231–1251 (2021).
6. Metz, M.-C. & Wiestler, B. Molecular imaging of gliomas. *Clin. Neuropathol.* **42**, 131–139 (2023).
7. Śledzińska, P., Bebyn, M. G., Furtak, J., Kowalewski, J. & Lewandowska, M. A. Prognostic and predictive biomarkers in gliomas. *Int. J. Mol. Sci.* **22**, 10373 (2021).
8. Di Nunno, V. et al. The biological and clinical role of the telomerase reverse transcriptase gene in glioblastoma: a potential therapeutic target? *Cells* **13**, 44 (2023).
9. Bianconi, A. et al. Updates in glioblastoma immunotherapy: an overview of the current clinical and translational scenario. *Biomedicines* **11**, 1520 (2023).
10. Saaid, A. et al. Prognostic analysis of the IDH1 G105G (rs11554137) SNP in IDH-wildtype glioblastoma. *Genes* **13**, 1439 (2022).
11. Jen, J. F. et al. Population pharmacokinetics of temozolomide in cancer patients. *Pharm. Res.* **17**, 1284–1289 (2000).
12. Zhang, J., Stevens, M. F. & Bradshaw, T. D. Temozolomide: mechanisms of action, repair and resistance. *Curr. Mol. Pharmacol.* **5**, 102–114 (2012).
13. Johannessen, T.-C. A. & Bjerkvig, R. Molecular mechanisms of temozolomide resistance in glioblastoma multiforme. *Expert Rev. Anticancer Ther.* **12**, 635–642 (2012).
14. Bianconi, A. et al. Effects of Levetiracetam and Lacosamide on survival and seizure control in IDH-wild type glioblastoma during temozolomide plus radiation adjuvant therapy. *Brain Spine* **4**, 102732 (2024).
15. Fisher, J. P. & Adamson, D. C. Current FDA-approved therapies for high-grade malignant gliomas. *Biomedicines* **9**, 324 (2021).
16. Suvà, M. L. & Tirosh, I. The glioma stem cell model in the era of single-cell genomics. *Cancer Cell* **37**, 630–636 (2020).
17. Beier, D. et al. CD133+ and CD133− glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Res.* **67**, 4010–4015 (2007).
18. Anido, J. et al. TGF-β receptor inhibitors target the CD44high/Id1high glioma-initiating cell population in human glioblastoma. *Cancer Cell* **18**, 655–668 (2010).
19. Son, M. J., Woolard, K., Nam, D.-H., Lee, J. & Fine, H. A. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. *Cell Stem Cell* **4**, 440–452 (2009).
20. Ogden, A. T. et al. Identification of A2B5+CD133− tumor-initiating cells in adult human gliomas. *Neurosurgery* **62**, 505–515 (2008).
21. He, J. et al. CD90 is identified as a candidate marker for cancer stem cells in primary high-grade gliomas using tissue microarrays. *Mol. Cell. Proteom.* **11**, M111.010744 (2012).
22. Lathia, J. D. et al. Integrin alpha 6 regulates glioblastoma stem cells. *Cell Stem Cell* **6**, 421–432 (2010).
23. Bao, S. et al. Targeting cancer stem cells through L1CAM suppresses glioma growth. *Cancer Res.* **68**, 6043–6048 (2008).
24. Chen, X. et al. The overexpression of epithelial cell adhesion molecule (EpCAM) in glioma. *J. Neurooncol.* **119**, 39–47 (2014).
25. Hemmati, H. D. et al. Cancerous stem cells can arise from pediatric brain tumors. *Proc. Natl. Acad. Sci.* **100**, 15178–15183 (2003).
26. Ben-Porath, I. et al. An embryonic stem cell–like gene expression signature in poorly differentiated aggressive human tumors. *Nat. Genet.* **40**, 499–507 (2008).
27. Tunici, P. et al. No title found]. *Mol. Cancer* **3**, 25 (2004).
28. Castellan, M. et al. Single-cell analyses reveal YAP/TAZ as regulators of stemness and cell plasticity in glioblastoma. *Nat. Cancer* **2**, 174–188 (2020).
29. Volnitskiy, A. et al. OCT4 expression in gliomas is dependent on cell metabolism. *Curr. Issues Mol. Biol.* **46**, 1107–1120 (2024).

30. Suvà, M. L. et al. Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. *Cell* **157**, 580–594 (2014).
31. Sanai, N., Alvarez-Buylla, A. & Berger, M. S. Neural stem cells and the origin of gliomas. *N. Engl. J. Med.* **353**, 811–822 (2005).
32. Rosenblum, M. L. et al. Age-related chemosensitivity of stem cells from human malignant brain tumours. *Lancet Lond. Engl.* **1**, 885–887 (1982).
33. Nomura, M. et al. The multilayered transcriptional architecture of glioblastoma ecosystems. *Nat. Genet.* **57**, 1155–1167 (2025).
34. Spitzer, A. et al. Deciphering the longitudinal trajectories of glioblastoma ecosystems by integrative single-cell genomics. *Nat. Genet.* **57**, 1168–1178 (2025).
35. Ayyadhury, S. et al. Glioblastoma stem cells show transcriptionally correlated spatial organization. *Commun. Biol.* **9**, 208 (2026).
36. Lu, C. et al. Combined targeting of glioblastoma stem cells of different cellular states disrupts malignant progression. *Nat. Commun.* **16**, 2974 (2025).
37. Bao, S. et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **444**, 756–760 (2006).
38. Ropolo, M. et al. Comparative analysis of DNA repair in stem and nonstem glioma cell cultures. *Mol. Cancer Res. MCR* **7**, 383–392 (2009).
39. Wu, J. et al. Knockdown of checkpoint kinase 1 is associated with the increased radiosensitivity of glioblastoma stem-like cells. *Tohoku J. Exp. Med.* **226**, 267–274 (2012).
40. Zhou, W. et al. Activation of the phosphorylation of ATM contributes to radioresistance of glioma stem cells. *Oncol. Rep.* **30**, 1793–1801 (2013).
41. Ahmed, S. U. et al. Selective inhibition of parallel DNA damage response pathways optimizes radiosensitization of glioblastoma stem-like cells. *Cancer Res.* **75**, 4416–4428 (2015).
42. Carruthers, R. et al. Abrogation of radioresistance in glioblastoma stem-like cells by inhibition of ATM kinase. *Mol. Oncol.* **9**, 192–203 (2015).
43. Raso, A. et al. Characterization of glioma stem cells through multiple stem cell markers and their specific sensitization to double-strand break-inducing agents by pharmacological inhibition of ataxia telangiectasia mutated protein. *Brain Pathol.* **22**, 677–688 (2012).
44. Cappelli, E., Vecchio, D. & Frosina, G. Delayed formation of FancD2 foci in glioma stem cells treated with ionizing radiation. *J. Cancer Res. Clin. Oncol.* **138**, 897–899 (2012).
45. Carruthers, R. D. et al. Replication stress drives constitutive activation of the DNA damage response and radioresistance in glioblastoma stem-like cells. *Cancer Res.* **78**, 5060–5071 (2018).
46. Lier, S. et al. CDK12/CDK13 inhibition disrupts transcriptional elongation and replication fork progression in glioblastoma. *EMBO Mol. Med.* https://doi.org/10.1038/s44321-026-00393-w (2026).
47. Li, J. et al. NAD+ bioavailability mediates PARG inhibition-induced replication arrest, intra S-phase checkpoint and apoptosis in glioma stem cells. *NAR Cancer* **3**, zcab044 (2021).
48. Hasegawa, D. et al. G-quadruplex ligand-induced DNA damage response coupled with telomere dysfunction and replication stress in glioma stem cells. *Biochem. Biophys. Res. Commun.* **471**, 75–81 (2016).
49. Kenig, S. et al. Topoisomerase IIβ mediates the resistance of glioblastoma stem cells to replication stress-inducing drugs. *Cancer Cell Int.* **16**, 58 (2016).
50. Pedersen, H., Anne Adanma Obara, E., Elbæk, K. J., Vitting-Serup, K. & Hamerlik, P. Replication Protein A (RPA) mediates radio-resistance of glioblastoma cancer stem-like cells. *Int. J. Mol. Sci.* **21**, 1588 (2020).
51. Osuka, S. et al. IGF1 receptor signaling regulates adaptive radioprotection in glioma stem cells. *Stem Cells* **31**, 627–640 (2013).
52. Mao, D. D., Cleary, R. T., Gujar, A., Mahlokozera, T. & Kim, A. H. CDC20 regulates sensitivity to chemotherapy and radiation in glioblastoma stem cells. *PLOS ONE* **17**, e0270251 (2022).
53. Lee, J.-K. et al. USP1 targeting impedes GBM growth by inhibiting stem cell maintenance and radioresistance. *Neuro-Oncol.* **18**, 37–47 (2016).
54. Tachon, G. et al. Cell cycle changes after glioblastoma stem cell irradiation: the major role of RAD51. *Int. J. Mol. Sci.* **19**, 3018 (2018).
55. Silvestre, D. C. et al. Alternative lengthening of telomeres in human glioma stem cells. *Stem Cells Dayt. Ohio* **29**, 440–451 (2011).
56. Berardinelli, F. et al. G-quadruplex ligand RHPS4 radiosensitizes glioblastoma xenograft in vivo through a differential targeting of bulky differentiated- and stem-cancer cells. *J. Exp. Clin. Cancer Res. CR* **38**, 311 (2019).
57. Rominiyi, O. et al. P04.74 Preclinical evaluation of combinations targeting the DNA damage response in 2D and 3D models of glioblastoma stem cells. *Neuro-Oncol.* **20**, iii297–iii297 (2018).
58. Hao, J. et al. The effects of extra high dose rate irradiation on glioma stem-like cells. *PLOS ONE* **13**, e0202533 (2018).
59. Marei, H. E., Pozzoli, G., Gaiba, A., Sonnessa, M. & Cenciarelli, C. Distinct molecular pathways leading to dosage-dependent temozolomide resistance in GBM stem cells. *Cancer Cell Int.* **26**, 118 (2026).
60. Mihalas, A. B. et al. KAT5 regulates neurodevelopmental states associated with G0-like populations in glioblastoma. *Nat. Commun.* **16**, 4327 (2025).
61. Liu, Y. et al. Autocrine endothelin-3/endothelin receptor B signaling maintains cellular and molecular properties of glioblastoma stem cells. *Mol. Cancer Res. MCR* **9**, 1668–1685 (2011).
62. Sharma, V., Dixit, D., Ghosh, S. & Sen, E. COX-2 regulates the proliferation of glioma stem like cells. *Neurochem. Int.* **59**, 567–571 (2011).
63. Hira, V. V. V. et al. Periarteriolar glioblastoma stem cell niches express bone marrow hematopoietic stem cell niche proteins. *J. Histochem. Cytochem.* **66**, 155–173 (2018).
64. Heffernan, J. M., McNamara, J. B., Vernon, B. L., Mehta, S. & Sirianni, R. W. PNJ scaffolds promote microenvironmental regulation of glioblastoma stem-like cell enrichment and radioresistance. *Biomater. Sci.* **10**, 819–833 (2022).
65. Lathia, J. D. et al. Laminin alpha 2 enables glioblastoma stem cell growth. *Ann. Neurol.* **72**, 766–778 (2012).
66. Cheng, L. et al. L1CAM regulates DNA damage checkpoint response of glioblastoma stem cells through NBS1. *EMBO J.* **30**, 800–813 (2011).
67. De Bacco, F. et al. MET inhibition overcomes radiation resistance of glioblastoma stem-like cells. *EMBO Mol. Med.* **8**, 550–568 (2016).
68. Stanzani, E. et al. Dual role of integrin alpha-6 in glioblastoma: supporting stemness in proneural stem-like cells while inducing radioresistance in mesenchymal stem-like cells. *Cancers* **13**, 3055 (2021).
69. Minata, M. et al. Multi-kinase inhibitor C1 triggers mitotic catastrophe of glioma stem cells mainly through MELK kinase inhibition. *PloS One* **9**, e92546 (2014).
70. Signore, M. et al. Combined PDK1 and CHK1 inhibition is required to kill glioblastoma stem-like cells in vitro and in vivo. *Cell Death Dis.* **5**, e1223 (2014).
71. Müller, A. et al. Concurrent activation of both survival-promoting and death-inducing signaling by chloroquine in glioblastoma stem cells: implications for potential risks and benefits of using chloroquine as radiosensitizer. *Cells* **12**, 1290 (2023).
72. Yuan, S. et al. Effective elimination of cancer stem cells by a novel drug combination strategy. *Stem Cells Dayt. Ohio* **31**, 23–34 (2013).
73. Ong, D. S. T. et al. PAF promotes stemness and radioresistance of glioma stem cells. *Proc. Natl. Acad. Sci. USA.* **114**, E9086–E9095 (2017).

74. Leong, V. W. S. et al. MGMT function determines the differential response of ATR inhibitors with DNA-damaging agents in glioma stem cells for GBM therapy. *Neuro-Oncol. Adv.* **6**, vdad165 (2024).
75. Jana, S. et al. PITAR, a DNA damage-inducible cancer/testis long noncoding RNA, inactivates p53 by binding and stabilizing TRIM28 mRNA. *eLife* **12**, RP88256 (2024).
76. Chen, H. et al. A brain-penetrating Hsp90 inhibitor NXD30001 inhibits glioblastoma as a monotherapy or in combination with radiation. *Front. Pharmacol.* **11**, 974 (2020).
77. Linder, B. et al. Arsenic trioxide and (-)-gossypol synergistically target glioma stem-like cells via inhibition of hedgehog and notch signaling. *Cancers* **11**, 350 (2019).
78. Yang, D. et al. High throughput-screening of native herbal compounds identifies taccaoside A as a cytotoxic compound that mediates RAS signaling in cancer stem cells. *Phytomed. Int. J. Phytother. Phytopharm.* **108**, 154492 (2023).
79. Sahu, U. et al. oHSV-P10 reduces glioma stem cell enrichment after oncolytic HSV therapy. *Mol. Ther. Oncolytics* **29**, 30–41 (2023).
80. Salvatori, L. et al. Nitric oxide prevents glioblastoma stem cells' expansion and induces temozolomide sensitization. *Int. J. Mol. Sci.* **24**, 11286 (2023).
81. Colapietro, A. et al. The botanical drug PBI-05204, a supercritical CO2 extract of nerium oleander, is synergistic with radiotherapy in models of human glioblastoma. *Front. Pharmacol.* **13**, 852941 (2022).
82. Alan Mitteer, R. et al. Proton beam radiation induces DNA damage and cell apoptosis in glioma stem cells through reactive oxygen species. *Sci. Rep.* **5**, 13961 (2015).
83. Morgenroth, A., Vogg, A. T. J., Ermert, K., Zlatopolskiy, B. & Mottaghy, F. M. Hedgehog signaling sensitizes glioma stem cells to endogenous nano-irradiation. *Oncotarget* **5**, 5483–5493 (2014).
84. Kanai, R. et al. Oncolytic virus-mediated manipulation of DNA damage responses: synergy with chemotherapy in killing glioblastoma stem cells. *J. Natl. Cancer Inst.* **104**, 42–55 (2012).
85. Jahan, N., Lee, J. M., Shah, K. & Wakimoto, H. Therapeutic targeting of chemoresistant and recurrent glioblastoma stem cells with a proapoptotic variant of oncolytic herpes simplex virus. *Int. J. Cancer* **141**, 1671–1681 (2017).
86. Gil-Ranedo, J., Gallego-García, C. & Almendral, J. M. Viral targeting of glioblastoma stem cells with patient-specific genetic and post-translational p53 deregulations. *Cell Rep.* **36**, 109673 (2021).
87. Yun, H. S. et al. The radiosensitizing effect of AZD0530 in glioblastoma and glioblastoma stem-like cells. *Mol. Cancer Ther.* **20**, 1672–1679 (2021).
88. Lazzarini, E. et al. Genome-wide profiling of patient-derived glioblastoma stem-like cells reveals recurrent genetic and transcriptomic signatures associated with brain tumors. *J. Neurooncol.* **163**, 47–59 (2023).
89. Reuss, D. E. et al. Simultaneous Nbs1 and p53 inactivation in neural progenitors triggers high-grade gliomas. *Neuropathol. Appl. Neurobiol.* **49**, e12915 (2023).
90. Vanderlinden, A., Jones, C. G., Myers, K. N., Rominiyi, O. & Collis, S. J. DNA damage response inhibitors enhance tumour treating fields (TTFields) potency in glioma stem-like cells. *Br. J. Cancer* https://doi.org/10.1038/s41416-023-02454-0 (2023).
91. Jones, C. G., Vanderlinden, A., Rominiyi, O. & Collis, S. J. Development and optimisation of tumour treating fields (TTFields) delivery within 3D primary glioma stem cell-like models of spatial heterogeneity. *Cancers* **16**, 863 (2024).
92. Jeitany, M. et al. A preclinical mouse model of glioma with an alternative mechanism of telomere maintenance (ALT). *Int. J. Cancer* **136**, 1546–1558 (2015).
93. Tentori, L. et al. Pharmacological inhibition of poly(ADP-ribose) polymerase-1 modulates resistance of human glioblastoma stem cells to temozolomide. *BMC Cancer* **14**, 151 (2014).
94. Shen, D. et al. Interferon-α/β enhances temozolomide activity against MGMT-positive glioma stem-like cells. *Oncol. Rep.* **34**, 2715–2721 (2015).
95. Patyka, M. et al. Sensitivity to PRIMA-1MET is associated with decreased MGMT in human glioblastoma cells and glioblastoma stem cells irrespective of p53 status. *Oncotarget* **7**, 60245–60269 (2016).
96. Gozdz, A. et al. Preservation of the hypoxic transcriptome in glioblastoma patient-derived cell lines maintained at lowered oxygen tension. *Cancers* **14**, 4852 (2022).
97. Sato, A. et al. MEK-ERK signaling dictates DNA-repair gene MGMT expression and temozolomide resistance of stem-like glioblastoma cells via the MDM2-p53 axis. *Stem Cells* **29**, 1942–1951 (2011).
98. Carrillo-Beltrán, D. et al. Glycosylated delphinidins decrease chemoresistance to temozolomide by regulating NF-κB/MGMT signaling in glioblastoma. *Cells* **14**, 179 (2025).
99. Wang, Y. et al. A biomimetic nanomedicine targets orthotopic glioblastoma by combinatorial co-delivery of temozolomide and a methylguanine-DNA methyltransferase inhibitor. *Adv. Ther.* **5**, 2200095 (2022).
100. Yun, H. S., Kramp, T. R., Palanichamy, K., Tofilon, P. J. & Camphausen, K. MGMT inhibition regulates radioresponse in GBM, GSC, and melanoma. *Sci. Rep.* **14**, 12363 (2024).
101. Wang, K. et al. siRNA nanoparticle suppresses drug-resistant gene and prolongs survival in an orthotopic glioblastoma xenograft mouse model. *Adv. Funct. Mater.* **31**, 2007166 (2021).
102. Lesueur, P. et al. Radiosensitization effect of talazoparib, a Parp Inhibitor, on glioblastoma stem cells exposed to low and high linear energy transfer radiation. *Sci. Rep.* **8**, 3664 (2018).
103. Huang, H. et al. lncRNA LUCAT1 regulates DNA damage response in glioma stem cells under hypoxia. *Neuro-Oncol*. noag017 https://doi.org/10.1093/neuonc/noag017(2026).
104. Schmid, R. S. et al. Core pathway mutations induce de-differentiation of murine astrocytes into glioblastoma stem cells that are sensitive to radiation but resistant to temozolomide. *Neuro-Oncol.* **18**, 962–973 (2016).
105. Steed, T. C. et al. Glioblastomas located in proximity to the subventricular zone (SVZ) exhibited enrichment of gene expression profiles associated with the cancer stem cell state. *J. Neurooncol.* **148**, 455–462 (2020).
106. Malacrida, A. et al. MV1035 overcomes temozolomide resistance in patient-derived glioblastoma stem cell lines. *Biology* **11**, 70 (2022).
107. Zhu, Y. et al. PDCD10 is a key player in TMZ-resistance and tumor cell regrowth: insights into its underlying mechanism in glioblastoma cells. *Cells* **13**, 1442 (2024).
108. Tang, J. & Yang, R. All-trans retinoic acid induces differentiation and downregulates stemness markers and MGMT expression in glioblastoma stem cells. *Cells* **14**, 746 (2025).
109. Kapoor-Narula, U. & Lenka, N. Elucidating the anti-tumorigenic efficacy of oltipraz, a dithiolethione, in glioblastoma. *Cells* **11**, 3057 (2022).
110. McCord, A. M., Jamal, M., Williams, E. S., Camphausen, K. & Tofilon, P. J. CD133+ glioblastoma stem-like cells are radiosensitive with a defective DNA damage response compared with established cell lines. *Clin. Cancer Res. J. Am. Assoc. Cancer Res.* **15**, 5145–5153 (2009).
111. Balbous, A. et al. A radiosensitizing effect of RAD51 inhibition in glioblastoma stem-like cells. *BMC Cancer* **16**, 604 (2016).
112. King, H. O. et al. RAD51 is a selective DNA repair target to radiosensitize glioma stem cells. *Stem Cell Rep.* **8**, 125–139 (2017).
113. Maachani, U. B. et al. FOXM1 and STAT3 interaction confers radioresistance in glioblastoma cells. *Oncotarget* **7**, 77365–77377 (2016).
114. Zheng, C. et al. Multiomics analyses reveal DARS1-AS1/YBX1-controlled posttranscriptional circuits promoting glioblastoma tumorigenesis/radioresistance. *Sci. Adv.* **9**, eadf3984 (2023).

115. Ding, J. et al. BRCA1 identified as a modulator of temozolomide resistance in P53 wild-type GBM using a high-throughput shRNA-based synthetic lethality screening. *Am. J. Cancer Res.* **9**, 2428–2441 (2019).
116. Onishi, S. et al. PRMT5 inhibition impairs Fanconi Anemia pathway-mediated homologous recombination and enhances the antitumor efficacy of Temozolomide in glioblastoma. *Cell Death Dis.* https://doi.org/10.1038/s41419-026-08739-5 (2026).
117. Russo, M. et al. MEOX2 enhances DNA repair and therapy resistance in Glioblastoma stem-like cells via PARP1 interaction. *Cancer Lett.* **642**, 218284 (2026).
118. Berte, N., Lokan, S., Eich, M., Kim, E. & Kaina, B. Artesunate enhances the therapeutic response of glioma cells to temozolomide by inhibition of homologous recombination and senescence. *Oncotarget* **7**, 67235–67250 (2016).
119. Ning, J., Wakimoto, H., Peters, C., Martuza, R. L. & Rabkin, S. D. Rad51 Degradation: Role in Oncolytic Virus-Poly(ADP-Ribose) Polymerase Inhibitor Combination Therapy in Glioblastoma. *J. Natl. Cancer Inst.* **109**, 1–13 (2017).
120. Ning, J.-F. et al. Myc targeted CDK18 promotes ATR and homologous recombination to mediate PARP inhibitor resistance in glioblastoma. *Nat. Commun.* **10**, 2910 (2019).
121. Mnatsakanyan, H. et al. SCD1 and SCD5 modulate PARP-dependent DNA repair via fatty acid desaturation in glioblastoma. *Cell Rep.* **45**, 117223 (2026).
122. Obara, E. A. A. et al. SPT6-driven error-free DNA repair safeguards genomic stability of glioblastoma cancer stem-like cells. *Nat. Commun.* **11**, 4709 (2020).
123. Król, S. K. et al. Aberrantly expressed RECQL4 helicase supports proliferation and drug resistance of human glioma cells and glioma stem cells. *Cancers* **12**, 2919 (2020).
124. Nakamura, T. et al. Targeting glioma stem cells in vivo by a G-quadruplex-stabilizing synthetic macrocyclic hexaoxazole. *Sci. Rep.* **7**, 3605 (2017).
125. Pinkham, K. et al. Stearoyl CoA desaturase is essential for regulation of endoplasmic reticulum homeostasis and tumor growth in glioblastoma cancer stem cells. *Stem Cell Rep.* **12**, 712–727 (2019).
126. Gomez-Roman, N. et al. Radiation responses of 2D and 3D glioblastoma cells: a novel, 3D-specific radioprotective role of VEGF/Akt signaling through functional activation of NHEJ. *Mol. Cancer Ther.* **19**, 575–589 (2020).
127. Xu, J. et al. Disruption of DNA repair and survival pathways through heat shock protein inhibition by onalespib to sensitize malignant gliomas to chemoradiation therapy. *Clin. Cancer Res. J. Am. Assoc. Cancer Res.* **28**, 1979–1990 (2022).
128. Fan, L. et al. UCHL3 induces radiation resistance and acquisition of mesenchymal phenotypes by deubiquitinating POLD4 in glioma stem cells. *Cell. Mol. Life Sci. CMLS* **81**, 247 (2024).
129. Zhou, X. et al. USP14 modulates stem-like properties, tumorigenicity, and radiotherapy resistance in glioblastoma stem cells through stabilization of MST4-phosphorylated ALKBH5. *Theranostics* **15**, 2293–2314 (2025).
130. Oldrini, B. et al. Somatic genome editing with the RCAS-TVA-CRISPR-Cas9 system for precision tumor modeling. *Nat. Commun.* **9**, 1466 (2018).
131. Zhuang, W. et al. Knockdown of the DNA-dependent protein kinase catalytic subunit radiosensitizes glioma-initiating cells by inducing autophagy. *Brain Res.* **1371**, 7–15 (2011).
132. Wang, Y. et al. Temporal DNA-PK activation drives genomic instability and therapy resistance in glioma stem cells. *JCI Insight* **3**, e98096 (2018).
133. Wang, W. et al. Knockdown of cathepsin L promotes radiosensitivity of glioma stem cells both in vivo and in vitro. *Cancer Lett.* **371**, 274–284 (2016).
134. Annovazzi, L. et al. The DNA damage/repair cascade in glioblastoma cell lines after chemotherapeutic agent treatment. *Int. J. Oncol.* **46**, 2299–2308 (2015).
135. Palanichamy, K. et al. Lack of constitutively active DNA repair sensitizes glioblastomas to Akt inhibition and induces synthetic lethality with radiation treatment in a p53-dependent manner. *Mol. Cancer Ther.* **17**, 336–346 (2018).
136. Sharifi, Z. et al. Mechanisms and antitumor activity of a binary EGFR/DNA-targeting strategy overcomes resistance of glioblastoma stem cells to temozolomide. *Clin. Cancer Res. J. Am. Assoc. Cancer Res.* **25**, 7594–7608 (2019).
137. Huang, J. et al. Dual targeting of DNA and EGFR by ZYH005 induces DNA damage and mitotic catastrophe in glioblastoma. *MedComm* **7**, e70717 (2026).
138. Mi, L. et al. Elevated nonhomologous end-joining by AATF enables efficient DNA damage repair and therapeutic resistance in glioblastoma. *Nat. Commun.* **16**, 4941 (2025).
139. Wen, N. et al. Bromodomain inhibitor jq1 induces cell cycle arrest and apoptosis of glioma stem cells through the VEGF/PI3K/AKT signaling pathway. *Int. J. Oncol.* https://doi.org/10.3892/ijo.2019.4863 (2019).
140. Wang, W. et al. NVP-BEZ235, a novel dual PI3K/mTOR inhibitor, enhances the radiosensitivity of human glioma stem cells in vitro. *Acta Pharmacol. Sin.* **34**, 681–690 (2013).
141. Wang, J. et al. Notch promotes radioresistance of glioma stem cells. *Stem Cells Dayt. Ohio* **28**, 17–28 (2010).
142. Riccitelli, E. et al. Extracellular sphingosine-1-phosphate: a novel actor in human glioblastoma stem cell survival. *PloS One* **8**, e68229 (2013).
143. Man, J. et al. Hyperthermia sensitizes glioma stem-like cells to radiation by inhibiting AKT signaling. *Cancer Res.* **75**, 1760–1769 (2015).
144. Yin, J. et al. Reactivating PTEN to impair glioma stem cells by inhibiting cytosolic iron-sulfur assembly. *Sci. Transl. Med.* **16**, eadg5553 (2024).
145. Xie, C. et al. GSCs differentiation model-informed nanotherapy: dual-functional brain-targeting liposomes with iRGD modification for co-delivery of osimertinib and bortezomib to combat radioresistant glioblastoma. *Cell Death Dis.* **16**, 738 (2025).
146. Visvanathan, A. et al. Essential role of METTL3-mediated m6A modification in glioma stem-like cells maintenance and radioresistance. *Oncogene* **37**, 522–533 (2018).
147. Shi, J. et al. METTL3 knockdown promotes temozolomide sensitivity of glioma stem cells via decreasing MGMT and APNG mRNA stability. *Cell Death Discov.* **9**, 22 (2023).
148. Zhou, D. et al. Distinctive epigenomes characterize glioma stem cells and their response to differentiation cues. *Genome Biol.* **19**, 43 (2018).
149. Zhang, J. et al. The m6A RNA demethylase FTO promotes radioresistance and stemness maintenance of glioma stem cells. *Cell. Signal.* **132**, 111782 (2025).
150. Kowalski-Chauvel, A. et al. The m6A RNA demethylase ALKBH5 promotes radioresistance and invasion capability of glioma stem cells. *Cancers* **13**, 40 (2020).
151. Alejo, S. et al. Lysine-specific histone demethylase 1A (KDM1A/LSD1) inhibition attenuates DNA double-strand break repair and augments the efficacy of temozolomide in glioblastoma. *Neuro-Oncol.* **25**, 1249–1261 (2023).
152. Staberg, M. et al. Targeting glioma stem-like cell survival and chemoresistance through inhibition of lysine-specific histone demethylase KDM 2B. *Mol. Oncol.* **12**, 406–420 (2018).
153. Ciechomska, I. A., Marciniak, M. P., Jackl, J. & Kaminska, B. Pre-treatment or post-treatment of human glioma cells with BIX01294, the inhibitor of histone methyltransferase G9a, sensitizes cells to temozolomide. *Front. Pharmacol.* **9**, 1271 (2018).

154. Yang, W., Liu, Y., Gao, R., Yu, H. & Sun, T. HDAC6 inhibition induces glioma stem cells differentiation and enhances cellular radiation sensitivity through the SHH/Gli1 signaling pathway. *Cancer Lett.* **415**, 164–176 (2018).
155. Luedi, M. M. et al. A dexamethasone-regulated gene signature is prognostic for poor survival in glioblastoma patients. *J. Neurosurg. Anesthesiol.* **29**, 46–58 (2017).
156. Rodrigues, P. M. G., De Souza, R. S., Borges, H. L. & Martins, R. A. P. Regulation of c-Myc and NBS1 by ionizing radiation in glioblastoma cells. *Oncol. Signal.* **1**, 1–5 (2018).
157. Wu, Q., Berglund, A. E., Macaulay, R. J. & Etame, A. B. Epigenetic activation of TUSC3 sensitizes glioblastoma to temozolomide independent of MGMT promoter methylation status. *Int. J. Mol. Sci.* **24**, 15179 (2023).
158. Li, F., Zhang, Y., Li, J., Jiang, R. & Ci, S. NUP98-p65 complex regulates DNA repair to maintain glioblastoma stem cells. *FASEB J. Publ. Fed. Am. Soc. Exp. Biol.* **39**, e70401 (2025).
159. Peruzzi, P. et al. MicroRNA-128 coordinately targets polycomb repressor complexes in glioma stem cells. *Neuro-Oncol.* **15**, 1212–1224 (2013).
160. Correa, B. R. et al. Functional genomics analyses of RNA-binding proteins reveal the splicing regulator SNRPB as an oncogenic candidate in glioblastoma. *Genome Biol.* **17**, 125 (2016).
161. Guardia, G. D. A. et al. Proneural and mesenchymal glioma stem cells display major differences in splicing and lncRNA profiles. *npj Genom. Med.* **5**, 2 (2020).
162. Yang, W. et al. MiR-146b-5p overexpression attenuates stemness and radioresistance of glioma stem cells by targeting HuR/lincRNA-p21/β-catenin pathway. *Oncotarget* **7**, 41505–41526 (2106).
163. Kosti, A. et al. Synergism of proneurogenic miRNAs provides a more effective strategy to target glioma stem cells. *Cancers* **13**, 289 (2021).
164. Cornelissen, F. M. G. et al. The translatome of glioblastoma. *Mol. Oncol.* **19**, 716–740 (2025).
165. Kahn, J. et al. The mTORC1/mTORC2 inhibitor AZD2014 enhances the radiosensitivity of glioblastoma stem-like cells. *Neuro-Oncol.* **16**, 29–37 (2014).
166. Wahba, A., Rath, B. H., Bisht, K., Camphausen, K. & Tofilon, P. J. Polysome profiling links translational control to the radioresponse of glioblastoma stem-like cells. *Cancer Res.* **76**, 3078–3087 (2016).
167. Wang, Y. et al. Lipolytic inhibitor G0S2 modulates glioma stem-like cell radiation response. *J. Exp. Clin. Cancer Res. CR* **38**, 147 (2019).
168. Wahba, A., Rath, B. H., O'Neill, J. W., Camphausen, K. & Tofilon, P. J. The XPO1 inhibitor selinexor inhibits translation and enhances the radiosensitivity of glioblastoma cells grown in vitro and in vivo. *Mol. Cancer Ther.* **17**, 1717–1726 (2018).
169. Shirakawa, Y. et al. Glioma cells acquire stem-like characters by extrinsic ribosome stimuli. *Cells* **10**, 2970 (2021).
170. Ye, F. et al. Protective properties of radio-chemoresistant glioblastoma stem cell clones are associated with metabolic adaptation to reduced glucose dependence. *PloS One* **8**, e80397 (2013).
171. Wang, L., Long, L., Wang, W. & Liang, Z. Resveratrol, a potential radiation sensitizer for glioma stem cells both in vitro and in vivo. *J. Pharmacol. Sci.* **129**, 216–225 (2015).
172. Kalluri, H. S. G., Kuo, J. S. & Dempsey, R. J. Effect of D609 on the expression of GADD45β protein: Potential inhibitory role in the growth of glioblastoma cancer stem like cells. *Eur. J. Pharmacol.* **791**, 510–517 (2016).
173. Li, Y. et al. Cytotoxic indole alkaloid 3α-acetonyltabersonine induces glioblastoma apoptosis via inhibition of DNA damage repair. *Toxins* **9**, 150 (2017).
174. Li, X. et al. Large-scale CRISPRi screens link metabolic stress to glioblastoma chemoresistance. *J. Transl. Med.* **23**, 289 (2025).
175. Marín-Ramos, N. I. et al. Inhibition of motility by NEO100 through the calpain-1/RhoA pathway. *J. Neurosurg.* **133**, 1020–1031 (2020).
176. Wu, S. et al. EGFR amplification induces increased DNA damage response and renders selective sensitivity to talazoparib (PARP Inhibitor) in glioblastoma. *Clin. Cancer Res. J. Am. Assoc. Cancer Res.* **26**, 1395–1407 (2020).
177. Godoy, P. R. D. V., Pour Khavari, A., Rizzo, M., Sakamoto-Hojo, E. T. & Haghdoost, S. Targeting NRF2, regulator of antioxidant system, to sensitize glioblastoma neurosphere cells to radiation-induced oxidative stress. *Oxid. Med. Cell. Longev.* **2020**, 2534643 (2020).
178. Tang, W. et al. Activation of chaperone-mediated autophagy suppresses glioblastoma by promoting wild-type IDH1/isocitrate dehydrogenase 1 degradation. *Autophagy* **22**, 245–265 (2026).
179. Li, G. et al. Glycometabolic reprogramming-induced XRCC1 lactylation confers therapeutic resistance in ALDH1A3-overexpressing glioblastoma. *Cell Metab.* **36**, 1696–1710.e10 (2024).
180. Liu, X. et al. PHGDH activation fuels glioblastoma progression and radioresistance via serine synthesis pathway. *J. Exp. Clin. Cancer Res. CR* **44**, 99 (2025).
181. Shi, Z. et al. *PGK1/PHGDH axis drives radioresistance glioblastoma stem cells* https://doi.org/10.2139/ssrn.6081070 (2026).
182. Eyme, K. M. et al. Targeting de novo lipid synthesis induces lipotoxicity and impairs DNA damage repair in glioblastoma mouse models. *Sci. Transl. Med.* **15**, eabq6288 (2023).
183. Majuelos-Melguizo, J. et al. Glioblastoma cells counteract PARP inhibition through pro-survival induction of lipid droplets synthesis and utilization. *Cancers* **14**, 726 (2022).
184. Evers, P. et al. Irradiation of the potential cancer stem cell niches in the adult brain improves progression-free survival of patients with malignant glioma. *BMC Cancer* **10**, 384 (2010).
185. Lemarié, A. et al. The STEMRI trial: magnetic resonance spectroscopy imaging can define tumor areas enriched in glioblastoma stem-like cells. *Sci. Adv.* **9**, eadi0114 (2023).
186. Riedel, A. et al. H2AX foci assay in glioblastoma: surgical specimen versus corresponding stem cell culture. *Radiother. Oncol. J. Eur. Soc. Ther. Radiol. Oncol.* **159**, 119–125 (2021).
187. Li, D. et al. Inhibiting macrophage-derived lactate transport restores cGAS–STING signalling and enhances antitumour immunity in glioblastoma. *Nat. Cell Biol.* **28**, 349–362 (2026).
188. Duan, T. et al. Nuclear cholesterol regulates nuclear size and DNA damage responses in cancer stem cells. *Neuro-Oncol.* **27**, 2530–2546 (2025).
189. Chaudhary, P. et al. Cellular irradiations with laser-driven carbon ions at ultra-high dose rates. *Phys. Med. Biol*. **68**, 387 (2023).
190. Huang, Z.-L. et al. Fractionated radiation therapy alters energy metabolism and induces cellular quiescence exit in patient-derived orthotopic xenograft models of high-grade glioma. *Transl. Oncol.* **45**, 101988 (2024).
191. Ganser, K. et al. KCa channel targeting impairs DNA repair and invasiveness of patient-derived glioblastoma stem cells in culture and orthotopic mouse xenografts which only in part is predictable by KCa expression levels. *Int. J. Cancer* **155**, 1886–1901 (2024).
192. Baugh, R. et al. Targeting NKG2D ligands in glioblastoma with a bispecific T-cell engager is augmented with conventional therapy and enhances oncolytic virotherapy of glioma stem-like cells. *J. Immunother. Cancer* **12**, e008460 (2024).
193. Ravi, V., Madhankumar, A. B., Abraham, T., Slagle-Webb, B. & Connor, J. R. Liposomal delivery of ferritin heavy chain 1 (FTH1) siRNA in patient xenograft derived glioblastoma initiating cells suggests different sensitivities to radiation and distinct survival mechanisms. *PLoS ONE* **14**, e0221952 (2019).
194. Wick, W. et al. Prognostic or predictive value of *MGMT* promoter methylation in gliomas depends on *IDH1* mutation. *Neurology* **81**, 1515–1522 (2013).

195. Brat, D. J. et al. Molecular biomarker testing for the diagnosis of diffuse gliomas. *Arch. Pathol. Lab. Med.* **146**, 547–574 (2022).
196. Van Den Bent, M. J. et al. Concurrent and adjuvant temozolomide for 1p/19q non-co-deleted anaplastic glioma (CATNON; EORTC study 26053-22054): final and exploratory analyses of a randomised, open-label, phase 3 trial. *Lancet Oncol.* **27**, 45–56 (2026).
197. Youssef, G. et al. Prognostic significance of O6-methylguanine-DNA methyltransferase promoter methylation status in isocitrate dehydrogenase-mutant glioma. *Neuro-Oncol.* **28**, 752–764 (2026).
198. Talele, S. et al. Brain distribution of berzosertib: an ataxia telangiectasia and Rad3-related protein inhibitor for the treatment of glioblastoma. *J. Pharmacol. Exp. Ther.* **379**, 343–357 (2021).
199. Lozinski, M. et al. ATR inhibition using gartisertib enhances cell death and synergises with temozolomide and radiation in patient-derived glioblastoma cell lines. *Oncotarget* **15**, 1–18 (2024).
200. Walter, B. et al. Functionally-instructed modifiers of response to ATR inhibition in experimental glioma. *J. Exp. Clin. Cancer Res.* **43**, 77 (2024).
201. Nitta, M. et al. Targeting EGFR induced oxidative stress by PARP1 inhibition in glioblastoma therapy. *PLoS ONE* **5**, e10767 (2010).
202. Schaff, L. et al. Phase II trial of combination pembrolizumab, olaparib, and temozolomide for patients with recurrent glioma. *J. Clin. Oncol.* **41**, TPS2087–TPS2087 (2023).
203. Majd, N. et al. Phase I clinical trial of peposertib plus radiation in adults with newly diagnosed MGMT-unmethylated glioblastoma. *J. Clin. Oncol.* **42**, 2076–2076 (2024).
204. Bright, S. J. et al. Targeted inhibition of DNA-PKcs, ATM, ATR, PARP, and Rad51 modulate response to X Rays and protons. *Radiat. Res*. **198**, 336–346 (2022).
205. Baker, J. H. E. et al. Radiation and chemo-sensitizing effects of DNA-PK inhibitors are proportional in tumors and normal tissues. *Mol. Cancer Ther.* **23**, 1230–1240 (2024).
206. Cheng, M. et al. YTHDF3 facilitates DNA damage response by recognizing METTL3-mediated m6A modification to promote chemotherapy resistance in glioblastoma. *Cancer Lett.* **649**, 218445 (2026).
207. Zhang, C. et al. METTL3 and N6-methyladenosine promote homologous recombination-mediated repair of DSBs by modulating DNA-RNA hybrid accumulation. *Mol. Cell* **79**, 425–442.e7 (2020).
208. Shi, J. et al. METTL3 promotes the resistance of glioma to temozolomide via increasing MGMT and ANPG in a m6A dependent manner. *Front. Oncol.* **11**, 702983 (2021).
209. Cui, Q. et al. m6A RNA methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells. *Cell Rep.* **18**, 2622–2634 (2017).
210. Faletti, S. et al. LSD1-directed therapy affects glioblastoma tumorigenicity by deregulating the protective ATF4-dependent integrated stress response. *Sci. Transl. Med.* **13**, eabf7036 (2021).
211. Agarwal, P. & Jackson, S. P. G9a inhibition potentiates the anti-tumour activity of DNA double-strand break inducing agents by impairing DNA repair independent of p53 status. *Cancer Lett.* **380**, 467–475 (2016).
212. Gursoy-Yuzugullu, O. et al. Epigenetic therapy with inhibitors of histone methylation suppresses DNA damage signaling and increases glioma cell radiosensitivity. *Oncotarget* **8**, 24518–24532 (2017).
213. Yang, Q. et al. G9a coordinates with the RPA complex to promote DNA damage repair and cell survival. *Proc. Natl. Acad. Sci. USA*. **114**, 6054–6063 (2017).
214. Tao, H. et al. Histone methyltransferase G9a and H3K9 dimethylation inhibit the self-renewal of glioma cancer stem cells. *Mol. Cell. Biochem.* **394**, 23–30 (2014).
215. Cao, Y. et al. G9a promotes immune suppression by targeting the Fbxw7/Notch pathway in glioma stem cells. *CNS Neurosci. Ther.* **29**, 2508–2521 (2023).
216. Mao, P. et al. Mesenchymal glioma stem cells are maintained by activated glycolytic metabolism involving aldehyde dehydrogenase 1A3. *Proc. Natl. Acad. Sci. USA* **110**, 8644–8649 (2013).
217. Islam, M. M. et al. Harnessing PHGDH inhibition for cancer therapy: mechanisms, SAR, computational aspects, and clinical potential. *Arch. Pharm.* **358**, e70083 (2025).
218. Zhang, D. et al. PHGDH-mediated endothelial metabolism drives glioblastoma resistance to chimeric antigen receptor T cell immunotherapy. *Cell Metab.* **35**, 517–534.e8 (2023).
219. Lassman, A. B. et al. A phase II study of the efficacy and safety of oral selinexor in recurrent glioblastoma. *Clin. Cancer Res.* **28**, 452–460 (2022).
220. Tang, Y. et al. Autophagy modulates glioblastoma cell sensitivity to Selinexor-mediated XPO1 inhibition. *Neuro-Oncol.* **27**, 1210–1226 (2025).
221. Mapunda, J. A. et al. XPO1-inhibitor Selinexor induces MGMT expression by activating PKA-CREB signaling in IDH wildtype glioblastoma. *Front. Oncol.* **15**, 1633580 (2025).
222. Odia, Y. et al. A phase 1/2 study of selinexor in combination with standard of care therapy for newly diagnosed or recurrent glioblastoma. *J. Clin. Oncol.* **39**, TPS2071–TPS2071 (2021).
223. Morgan, M. A. & Canman, C. E. Replication stress: an Achilles' Heel of glioma cancer stem–like cells. *Cancer Res.* **78**, 6713–6716 (2018).
224. Struve, N. et al. Increased replication stress and R-loop accumulation in EGFRvIII-expressing glioblastoma present new therapeutic opportunities. *Neuro-Oncol. Adv.* **4**, vdab180 (2022).
225. Helmrich, A., Ballarino, M. & Tora, L. Collisions between replication and transcription complexes cause common fragile site instability at the longest human genes. *Mol. Cell* **44**, 966–977 (2011).
226. García-Muse, T. & Aguilera, A. Transcription-replication conflicts: how they occur and how they are resolved. *Nat. Rev. Mol. Cell Biol.* **17**, 553–563 (2016).
227. Daniel, L. et al. Mechanistic insights in transcription-coupled nucleotide excision repair of ribosomal DNA. *Proc. Natl. Acad. Sci*. USA. **115**, 6770–6779 (2018).
228. Walker, F. M. et al. Rapid P-TEFb-dependent transcriptional reorganization underpins the glioma adaptive response to radiotherapy. *Nat. Commun.* **15**, 4616 (2024).
229. Tornaletti, S. & Hanawalt, P. C. Effect of DNA lesions on transcription elongation. *Biochimie* **81**, 139–146 (1999).
230. Hanawalt, P. C. Transcription-coupled repair and human disease. *Science* **266**, 1957–1958 (1994).
231. Hanawalt, P. C. & Spivak, G. Transcription-coupled DNA repair: two decades of progress and surprises. *Nat. Rev. Mol. Cell Biol.* **9**, 958–970 (2008).
232. Ward, J. F. DNA Damage Produced by Ionizing Radiation in Mammalian Cells: Identities, Mechanisms of Formation, and Reparability. In: *Proc. Progress in Nucleic Acid Research and Molecular Biology* vol. 35, 95–125 (Elsevier, 1988).
233. Cadet, J., Douki, T., Gasparutto, D. & Ravanat, J.-L. Oxidative damage to DNA: formation, measurement and biochemical features. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* **531**, 5–23 (2003).
234. Ljungman, M. & Lane, D. P. Transcription—guarding the genome by sensing DNA damage. *Nat. Rev. Cancer* **4**, 727–737 (2004).
235. Jia, N. et al. Dealing with transcription-blocking DNA damage: repair mechanisms, RNA polymerase II processing and human disorders. *DNA Repair* **106**, 103192 (2021).
236. Llerena Schiffmacher, D. A., Pai, Y. J., Pines, A. & Vermeulen, W. Transcription-coupled repair: tangled up in convoluted repair. *FEBS J.* **292**, 5288–5323 (2025).
237. Kim, J. E. et al. PHF8 facilitates transcription recovery following DNA double-strand break repair. *Nucleic Acids Res.* **52**, 10297–10310 (2024).
238. Donnio, L.-M. et al. XAB2 dynamics during DNA damage-dependent transcription inhibition. *eLife* **11**, e77094 (2022).

239. Donnio, L. & Giglia-Mari, G. Keep calm and reboot – how cells restart transcription after DNA damage and DNA repair. *FEBS Lett.* **599**, 275–294 (2025).
240. van der Woude, M., Davó-Martínez, C., Thijssen, K. L., Vermeulen, W. & Lans, H. Recovery of protein synthesis to assay DNA repair activity in transcribed genes in living cells and tissues. *Nucleic Acids Res* **51**, e93–e93 (2023).
241. Villalva, C. et al. O6-methylguanine-methyltransferase (MGMT) promoter methylation status in glioma stem-like cells is correlated to temozolomide sensitivity under differentiation-promoting conditions. *Int. J. Mol. Sci.* **13**, 6983–6994 (2012).
242. Jeong, D. E. et al. Preclinical and clinical implications of TERT promoter mutation in glioblastoma multiforme. *Oncol. Lett.* https://doi.org/10.3892/ol.2017.7196 (2017).
243. Liffers, K., Lamszus, K. & Schulte, A. *EGFR* amplification and glioblastoma stem-like cells. *Stem Cells Int.* **2015**, 1–11 (2015).

Acknowledgements

This work was supported by grants from Italian Ministry of Health - 5 ×1000 funds 2021 and by the Guido Berlucchi Foundation Mini Grant (C33C25001330007).

Author contributions

N.C.M., V.L., A.B. and E.C. researched the data to include in the review article. N.C.M., V.L., A.B. and E.C. wrote the first draft of the manuscript. D.D.P., D.G. and E.C. contributed to the conception and design of figures. N.C.M., A.B., P.F. and E.C. reviewed and edited material prior to submission. E.C. supervised and contributed to discussions of content.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s42003-026-10467-7.

Correspondence and requests for materials should be addressed to Elena Cerutti.

Peer review information *Communications Biology* thanks the anonymous reviewers for their contribution to the peer review of this work. Primary Handling Editors: Joanna Timmins and Kaliya Georgieva. A peer review file is available.