Review

DNA Damage Response and Growth Factor Signaling Pathways in Gliomagenesis and Therapeutic Resistance

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Abstract

The dismal prognosis of glioblastoma multiforme (GBM) is mainly due to the poor response of GBM patients to any therapeutic modalities, which include ionizing radiation and DNA-alkylating agents. In the last few years, the important role of the DNA damage response (DDR) pathway in tumor formation and modulation of therapeutic response has been appreciated. Interestingly, several of the genetic alterations commonly found in GBMs (such as epidermal growth factor receptor amplification and PTEN inactivation) have also recently been shown to regulate the activity of the DNA repair machinery and, consequently, the response to DNA-damaging agents used routinely in the clinic. In this review, we focus on some of these findings that suggest that at least some of the pathways driving GBM formation could be directly responsible for the therapy resistance of this tumor type. Possible therapeutic approaches exist that may either overcome or take advantage of these GBM genetic alterations to improve the response of these tumors to DNA-damaging therapy. *Cancer Res; 71(18); 1–5. ©2011 AACR.*

Introduction

Glioblastoma multiforme (GBM) is the most frequent cancer of the central nervous system in adults and is among the most aggressive and lethal tumor types. GBM patients routinely undergo maximal tumor mass resection followed by concurrent radiotherapy and chemotherapy, using the alkylating agent temozolomide (TMZ). Despite decades of research efforts, GBM patients remain refractory to current treatment modalities and have a dismal prognosis. Gaining insights into the pathways that determine this poor response to therapy will be instrumental for the development of new therapeutic modalities. Resistance to radiation and chemotherapy characterizes many cancer types; however, it is not clear whether resistance is acquired during tumor progression or if it is intrinsically associated with the genetic events that lead to the tumor formation in the first place. In this review, we highlight some of the recent findings, which suggest that the genetic alterations characterizing GBM genomes might also be responsible for the poor treatment response of this tumor type by directly modulating the activity of the DNA damage response (DDR), namely, the DNA damage checkpoint and the DNA repair machinery. This "DDR-centric" view of glioma biology and response to therapy may provide additional insight into this particularly vexing problem. Cur-

rently, several laboratories are exploring the possibility of manipulating the DDR to cause selective tumor cell death through catastrophic genomic instability; therefore, we also focus here on some of the possible therapeutic modalities that could overcome and/or exploit GBM genetic alterations to improve the response to radiotherapy and chemotherapy.

In response to DNA damage, cells activate the phosphoinositide 3-kinase (PI3K)–related kinases (PI3KK) ATM, ATR, and DNA-dependent protein kinase (DNA-PK), which in turn phosphorylate multiple downstream substrates, including the effector kinases Chk1 and Chk2, resulting in cell-cycle checkpoint initiation and/or apoptosis. The activation of DDR signaling also requires several accessory proteins known as checkpoint mediators or adaptors, including 53BP1, BRCA1, and MDC1. Recently, it has been proposed that the DDR acts as a barrier against tumor progression, in which early malignant lesions have to inactivate p53 or other components of the DDR to progress to a more aggressive status.

The genes encoding components of the ATM/Chk2 and the ATR/Chk1 pathways are subjected to frequent copy-number loss in GBM patients (Fig. 1), with heterozygous loss of *CHEK2* being the most frequent event (approximately 20%; ref. 1). Somatic cell gene transfer with the RCAS/tv-a system and platelet-derived growth factor (PDGF)–induced glioma generation have been used to show that some of the essential molecules of the DDR, such as ATM, Chk2, and p53, are required for glioma tumor suppression in mice and that loss of any of those genes not only accelerates tumor formation but also leads to a more aggressive phenotype, increasing the frequency of high-grade tumors (GBMs). Moreover, Chk2-null gliomas show defects in both apoptotic response and cell-cycle checkpoints, which prevent an ionizing radiation (IR)–mediated survival benefit observed in control mice. The evidence points to an indispensible role of Chk2 in IR response

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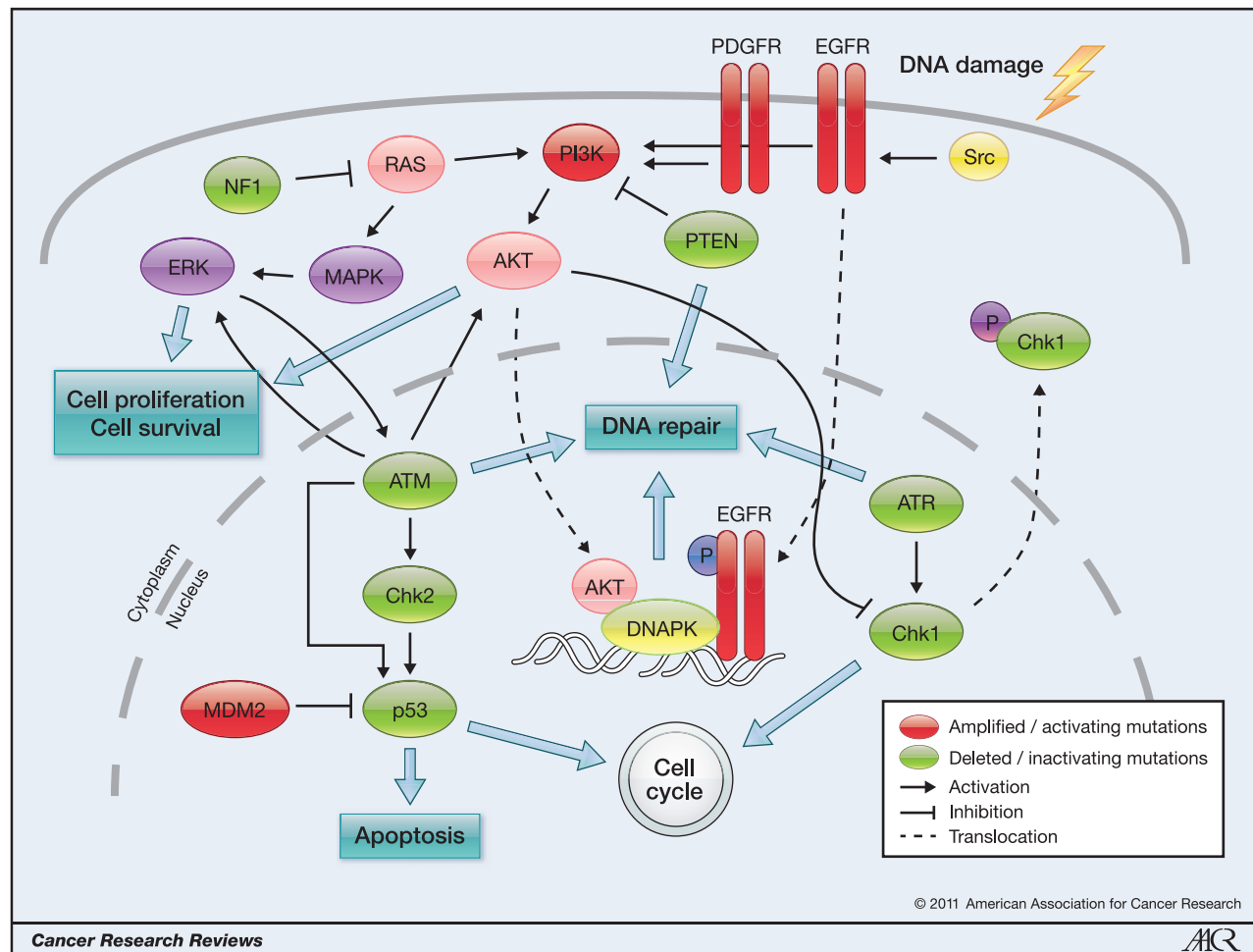
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Figure 1. Multiple signaling pathways altered in GBM tumors modulate the DDR. Genes with copy-number loss and/or inactivating mutations or copy-number gain and/or activating mutations are presented in green or red, respectively. See text for details.

in gliomas, but it may not necessarily be directly extended to other molecules of the DDR pathway, because of the intricacy of this signaling pathway. Inhibition of the ATM kinase (the upstream Chk2 activator that acts in concert and in parallel with Chk2 in DNA-damage checkpoint modulation) leads to radiosensitization of human glioma cells *in vitro* (2, 3). Given the vast collection of proteins that are phosphorylated by ATM (more than 700), it is very difficult to define which of its downstream targets mediates the increased sensitivity to IR in cells treated with an ATM inhibitor. However, one likely function of ATM that contributes to this phenotype is its ability to modulate the double-strand break (DSB) repair via homologous recombination (HR; ref. 3). DSBs are the most toxic DNA lesions, and failure to repair them could result in the loss of genetic information and the generation of danger-ous genomic rearrangements, which may ultimately lead to cell death. Treatment of the U87 glioma cell line with caffeine or more specific ATM inhibitors (KU-55933 and KU-60019) significantly reduced HR efficiency (2–4) and also affected AKT- and extracellular signal regulated kinase (ERK)–mediated prosurvival signaling and cell migration and/or

invasion (2). However, the efficacy of pharmacologic inhibition of ATM in combination with IR remains to be evaluated on GBMs *in vivo*. Moreover, p53 status is likely to determine the response of such a therapeutic strategy, with p53-functional tumors being resistant and the p53-mutant tumors being sensitive to ATM inhibition (5).

The alkylating agent TMZ is administered to GBM patients concurrently with radiotherapy. TMZ leads to the formation of a wide spectrum of methyl adducts typically represented by *N*-methylpurines, which are rapidly repaired by base excision repair (BER). The cytotoxicity of TMZ is thought to be mediated mainly by the formation of O^6-methylguanine (O^6-meG) DNA adducts. These methyl groups are normally removed by the methyl guanine methyl transferase (MGMT) enzyme, whereas the unrepaired O^6-meGs trigger a futile mismatch repair (MMR) cycle, which eventually leads to DSB formation that induces the activation of both ATM- and ATR-mediated signaling (6). A very prominent effect of TMZ treatment in glioma cells *in vitro* is a Chk1- and Chk2-dependent G_2 to M–cell-cycle arrest (7, 8). It has been shown that Chk2 gene silencing prevents the TMZ-induced G_2 arrest

(8), whereas ATR gene silencing or pharmacologic inhibition of Chk1 increases TMZ sensitivity (7, 9). The most common mechanism of TMZ resistance in GBM patients is MGMT expression, and *MGMT* promoter silencing by methylation corresponds to a better therapeutic response to TMZ. A deeper knowledge of the signaling pathways activated by TMZ treatment and how the DNA damage induced by TMZ treatment is repaired will be required to increase its therapeutic efficacy. Inhibition of PARP, a key component of BER, represents an attractive treatment approach in combination with different chemotherapy agents, including TMZ, and it is currently showing promising potential as monotherapy in HR-deficient tumors (such as BRCA-mutant breast cancer cells). Various PARP inhibitors are in phase I and II clinical trials at the moment, and several of these inhibitors (for example ABT-888, CEP-6800, AG014699, and GP15427) have been successfully used in preclinical mouse xenograft glioma models (10).

The epidermal growth factor receptor (EGFR) is one of the most frequently altered receptor tyrosine kinases (RTK) in GBM patients. It is amplified in approximately 40% of GBM samples overall and in 80% of GBMs of the classical subtype, and the variant III deletion of the extracellular domain (EGFR vIII mutant), the most commonly described event that leads to EGFR activation, is present in about half of these EGFR-amplified tumors. RTK signaling hyperactivation seems to be one of the initial oncogenic events in the majority of GBM, most probably largely mediated through the PI3K/Akt/mTOR and Ras/mitogen-activated protein kinase (MAPK) downstream signaling pathway (11). Various studies have shown that the activation of the PI3K/Akt and Ras/MAPK signaling pathways in cancer cells is significantly associated with radiotherapy resistance, either through the modulation of cell survival signaling or, most importantly for the focus of this review, by direct regulation of the DNA repair machinery.

The initial evidence that indicated a possible role of EGFR in regulation of DNA repair was published more than a decade ago (12), and it showed the direct interaction between EGFR and DNA-PK, one of the key components of the nonhomolgous end-joining (NHEJ) machinery. This initial work was extended in the context of radiation treatment with reports showing that both IR and cisplatin induces the translocation of EGFR into the nucleus, where it interacts with DNA-PK and, consequently, results in increased DNA-PK activity (13). This nuclear import of EGFR was inhibited by preincubation with the C225 monoclonal antibody (cetuximab), with a consequent radiosensitization of both lung (A549) and breast (MDA MB231) cancer cell lines, as measured by clonogenic-forming ability and resolution of γH2AX IR-induced foci (IRIF), a standard marker of DSBs (14). Analogously, cetuximab enhanced *in vitro* and, more importantly, *in vivo* radiosensitivity in squamous cell carcinoma (SCC) cells of the head and neck (15, 16). Moreover, EGFR inhibition by gefinitib treatment leads to a synergistic increase in growth inhibition when combined with other DNA-damaging agents (cisplatin and etoposide), rather than IR, in a human breast cancer cell line (MCF-7; ref. 17). When studied in more detail, EGFR signaling seems to positively regulate both HR and NHEJ in U87 glioma cells (18), and treatment with EGF ligand induces a dose-dependent increase in HR and NHEJ, which is reduced to 50% of basal levels in the presence of a specific EGFR inhibitor (AG1478). Similarly, activation of EGFR signaling by the expression of EGFR vIII mutant leads to more rapid resolution of γH2AX IRIF and an increase in DNA repair efficiency, whereas the opposite results are obtained when EGFR is inhibited by the EGFR-CD533 dominant-negative mutant. In sum, the results are consistent with the argument that DSB repair is regulated at multiple levels by growth factor signaling and that modulation of DNA repair by EGFR vIII might contribute to the radioresistance of GBMs that carry this mutation. In further support of this idea, a recent report (19) has shown that EGFR vIII expression in mouse astrocytes and human glioma cells (U87) leads to radioresistance by promoting DNA-PK activation and DSB repair, perhaps as a consequence of hyperactivated PI3K/Akt signaling. Mouse orthotopic tumors expressing EGFR vIII are refractory to radiation therapy, continuing to grow after whole-brain radiation, with little effect on overall survival. In U87 cells expressing EGFR vIII, blocking the receptor with the anti-EGFR monoclonal antibody mab-806 reduces the radioresistance of these glioma cells and leads to a reduction of tumor growth, with a concomitant decrease in the tumor microvascular density (20).

Taken together, the data discussed here suggest that EGFR signaling, either directly through the interaction with the DNA repair machinery or indirectly through the activation of PI3K/Akt and Ras/MAPK signaling pathways (Fig. 1), modulates sensitivity to radiation. Therefore, combining radiotherapy with inhibition of EGFR signaling might achieve a better therapeutic outcome, at least in the subset of glioma patients in which this signaling is activated.

Loss of function of the tumor suppressor gene PTEN, mainly by gene deletion or mutation, is a very frequent genetic aberration in GBM patients (approximately 35%). In addition to the well-established role of PTEN in the modulation of the PI3K/Akt/mTOR signaling pathway by the regulation of the intracellular levels of phosphatidylinositol 3,4,5-trisphosphate, PTEN exerts an essential role in maintaining chromosomal integrity via its ability to regulate centromere stability (in a phosphatase-independent manner), DNA DSB repair (possibly through the regulation of Rad51 expression), and Chk1 localization (21). PTEN regulation of HR results in defects in Rad51 foci formation and DSB repair. Comparing PTEN null cells with their isogenic cell lines has shown that the HR deficiency caused by PTEN loss sensitizes tumor cells to PARP inhibitors both *in vitro* and *in vivo* (22, 23). Analogously, PTEN-null astrocytes, as well as human glioma lines, have a compromised HR pathway (because of reduced levels of Rad51 paralogs), which confers sensitivity to N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), a functional analog of TMZ, and renders them sensitive to the PARP inhibition (24). However, at the same time, PTEN loss strongly activated PI3K signaling and resulted in increased resistance to IR, as assayed by colony survival (24, 25). Therefore, PTEN status *per se* will not be sufficient to predict therapy response in GBM patients because IR and TMZ are used concomitantly.

The signaling abnormalities that drive the other main GBM subsets include PDGF receptor (PDGFR; in the case of the proneural GBMs) and loss of the Ras negative regulator neurofibromatosis type 1 (NF1) gene (in the case of the mesenchymal GBMs; ref. 11). Although less is known about the connection between these pathways and the DDR, some data link them. The PDGF signaling drives tumor formation in a subset of GBMs (approximately 25%), determined either by genetic alterations (such as gene amplification, intrachromosomal deletion, and activating point mutation) of the PDGFRA gene or by overexpression the PDGF ligand (26). Even though direct modulation (like the one observed for EGFR) of the DNA repair machinery by PDGFR has not been described to date, various reports show that treatment with the PDGFR inhibitor imatinib increases radiation sensitivity in glioma cells *in vitro* and in glioma xenograft mouse models *in vivo* (27). As mentioned previously in this review, activation of the Ras/MAPK signaling pathways in cancer cells has been associated with radiotherapy resistance; however, it is still unknown whether NF1 inactivation in GBM patients affects the DNA damage checkpoints and/or DNA repair and, ultimately, the treatment response to DNA-damaging agents.

Concluding Remarks

In this review, we have taken the view that the DDR is a central component in the pathways that lead to glioma formation and progression. This DDR-centric view of the world, although clearly biased toward the role of this pathway in glioma biology, provides an alternative view of the connections between these known drivers of tumor formation and assumed contributors to therapeutic resistance. In so doing, we feel that insight to the system as a whole is provided. We have discussed how some of the genetic alterations that characterize the GBM genome can modulate, positively and/or negatively, the response to a given DNA-damaging agent (such as IR or TMZ), and it seems that a balance between the cell survival signals and an effective DNA repair will determine the ultimate outcome to a specific therapy. The DDR is a very convoluted signaling pathway, composed of several hundred proteins, few of which are altered in GBM patients (ref. 1; M. Squatrito and colleagues, manuscript in preparation). A better understanding of the molecular mechanisms that protect cells from the response to a specific DNA-damaging therapy is required to develop new treatments that could overcome this resistance.

It is important to keep in mind that GBM is a very heterogeneous disease composed of multiple subtypes characterized by distinctive genetic alterations that lead to the activation of different signaling pathways. These subtypes are likely to respond in different ways to a given therapy. As an example, the mesenchymal GBM subclass seems more likely to respond to radiotherapy than to alkylating agents (BCNU or TMZ), whereas classical GBM are more prone to respond to alkylating agents than to radiotherapy (28). In addition, any GBM is composed of multiple cell types that are likely to have a heterogeneous sensitivity to any specific treatment. For instance, GBM stem-like cells have been shown to be more radioresistant because of their ability to repair the radiation-induced DNA damage more effectively (29), although this initial evidence has recently been challenged (30). Moreover, similar to what is seen in SHH-driven medulloblastoma, cells that reside in the so-called perivascular niche of PDGF-induced gliomas (some of which seem to have stem-like characteristics) seem to be more resistant to IR.

The studies discussed in this review suggest that there is a strong link between the pathways involved in the process of gliomagenesis and the tumor's resistance to the DNA-damaging agents routinely used in the clinic (such as IR and TMZ). Given the intricate link between these 2 complex biological pathways, it is not surprising that these tumors are highly resistant to the standard cancer treatments that rely on DNA damage. Moreover, future therapeutic strategies for these tumors will need to take into account the effects of the pathways that drive the tumors on the characteristics that make them inherently resistant to therapy.

Disclosure of Potential Conflicts of Interest

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