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.

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.
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 \textit{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 dangerous 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 \textit{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 O6-methylguanine (O6-meG) DNA adducts. These methyl groups are normally removed by the methyl guanine methyl transferase (MGMT) enzyme, whereas the unrepaired O6-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 \textit{in vitro} is a Chk1- and Chk2-dependent G2–cell-cycle arrest (7, 8). It has been shown that Chk2 gene silencing prevents the TMZ-induced G2 arrest.

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.
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(C8), 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 mono-therapy 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 nonhomologous 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). 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.

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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 PDGFR 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 PDGFRα 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

No potential conflicts of interest were disclosed.

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