Letter to the Editor

Hidden in plain sight: promising therapeutic targets for glioblastoma lurk within DNA damage response pathways

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In Dr. Herst's excellent "Perspective", the author reflects on recent attempts to improve outcomes for patients with glioblastoma (GBM) by combining conventional cytotoxic agents (radiation therapy and temozolomide) with molecular targeted agents. We agree with her conclusion that targeting epidermal growth factor receptor (EGFR) signalling and angiogenesis has been unsuccessful to date, despite the apparently sound scientific rationale and promising pre-clinical data. We found her argument that components of the DNA damage response (DDR) represent more promising therapeutic targets to be very persuasive, and value the opportunity to respond to some of the key points.

It is interesting to consider why so few agents targeting the DDR have yet undergone clinical evaluation in GBM. A likely factor is the paucity of references to DDR genes in the landmark publications from The Cancer Genome Atlas Research Network, which comprehensively described the landscape of genomic abnormalities in GBM (1,2). Apart from the p53 and retinoblastoma (RB) pathways, which harboured genetic amplifications, deletions or mutations in the majority of tumours, the key genetic components of the DDR were conspicuous by their absence from these reports.

On initial consideration these findings are incompatible with high quality immunohistochemical studies of glioma which have revealed aberrant and constitutive upregulation and activation of DNA damage signalling, particularly in GBM. Bartkova and colleagues proposed that DDR activation occurs early in gliomagenesis and initially acts to repress the proliferative effects of oncogenic signalling pathways (3). They argued that loss of cell cycle checkpoint integrity (primarily G1/S) is responsible for transformation of low grade tumours to higher grades. This theory implies increased dependency of GBM cell survival on the G2-M checkpoint, a model that is supported by the constitutive upregulation and activation (phosphorylation) of ataxia telangiectasia mutated (ATM), Chk1 and Chk2 that they observed in GBM specimens. These observations can be reconciled with the genomic data by considering the findings of Squatrito and colleagues who demonstrated tumour suppressor functions of DDR components including ATM, Chk2 and p53 in genetic mouse models of GBM, and highlighted relatively frequent copy number alterations in these genes in the TCGA GBM data set (4).

It is also important to bear in mind that DDR phenotypes reflect integrity and function of pathways rather than individual genes. Furthermore, the requirement for a rapid cellular response to DNA damage means that protein phosphorylation biomarkers are generally of greater significance than gene expression readouts (5). This provides another explanation for the apparent absence of DDR genes from the published landscape of genomic abnormalities in GBM, and indicates that potential biomarkers predicting benefit from the addition of DDR inhibitors to conventional treatment are likely to involve readouts of pathway function rather than simple assays of gene expression or mutation.

In the Perspective, Herst describes the strategy of DDR targeting as "cell cycle checkpoint abrogation". We propose that the DDR comprises both cell cycle checkpoints and DNA repair functions, and that components of both networks are potential therapeutic targets. Repair of radiation induced DNA double strand breaks (DSB) is mediated by non-homologous end joining (NHEJ)

and homologous recombination (HR) and there is some evidence that tumours in general and GBM in particular are more dependent on HR repair than the adjacent normal tissues (6). Hence a number of research groups are working to develop specific chemical inhibitors of HR, but no clinical candidates have emerged to date. It is our view that combining radiation therapy (or chemoradiation) with inhibition of NHEJ would be counterintuitive, since the normal cells of the brain are predominantly non-dividing and hence likely to be strongly dependent on NHEJ for DSB repair (7). Radiation induced single strand breaks (SSB) are repaired predominantly by the base excision repair pathway (BER), within which poly(ADP-ribose) polymerase-1 (PARP-1) is the most highly developed target (8). Of the various PARP-1 inhibitors in clinical use, both veliparib and olaparib are being evaluated in the treatment of GBM. Initial studies combining veliparib with temozolomide, either alone or in combination with radiation, were discontinued because of haematological toxicity. We are currently undertaking a number of early phase clinical trials combining olaparib with radiation and/or temozolomide in a variety of different GBM populations and the early results indicate that these combinations are well tolerated.

Herst identifies ATM as a particularly promising target in GBM. We concur with this view and have published evidence that the potent radiosensitising effects of ATM inhibition on GBM stem-like cells are mediated by simultaneous abrogation of both the cell cycle checkpoint and DNA repair functions of this critical DDR protein (9,10). Herst cites extensive cellular and xenograft data to support the notion that ATM inhibition significantly enhances the tumoricidal effects of radiation both *in vitro* and *in vivo*. While we concur with the overall conclusion, we would like to challenge the inference that the radiosensitising effects of ATM inhibitors are observed only in p53 mutant cell lines. Using clonogenic survival assays, we observed potent sensitization of three primary GBM cell cultures (E2, R10 and G7) by KU-55933, of which E2 and G7 were shown by Sanger sequencing of exons 3–10 to be p53 wild type (R10 not sequenced to date). Whilst acknowledging the clear impact of p53 mutation on the radiosensitising effects of KU-60019, which is demonstrated by the elegant orthotopic experiments performed by Biddlestone-Thorpe and colleagues (11), we believe that, in this context, it is important to distinguish between p53 mutation and G1-S cell cycle checkpoint integrity. TCGA data show that, while p53 mutation or deletion is observed in only 35% of GBM specimens, abnormalities in the p53 signalling pathway are detected in 87% of tumours (1). Furthermore, genetic alterations in the RB signalling pathway, which is integral to G1-S checkpoint function, were observed in 78% of specimens. We therefore theorize that the lack of radiosensitization observed in p53 wild type U87 xenografts indicates retention of G1-S integrity, which is an atypical GBM phenotype. In this scenario, it is entirely plausible that functional G1-S arrest protects tumour cells from the impact of ATM inhibition on G2-M checkpoint function. In our studies, all three primary cell cultures failed to activate G1 arrest in response to radiation, despite the intact p53 genotype. These observations support the concept that functional readouts of DDR integrity are likely to be more beneficial as predictive biomarkers than individual genotypic features.

The critical question of normal tissue toxicity is also raised. Current "standard of care" for GBM (60 Gy to gross tumour volume plus a 2–3 cm margin in all dimensions, with concomitant and adjuvant temozolomide) is associated with an appreciable risk of neurocognitive toxicity which, if it occurs, is irreversible and often devastating. While the magnitude of the radiosensitising effect of ATM inhibition on tumour cells raises concerns over the possibility of increasing the likelihood and/or exacerbating the severity of neurological toxicity, there are grounds for cautious optimism. Firstly, *in vitro* studies performed by Golding demonstrated no impact of KU-60019 on radiation effects on astrocytes (12). While Vecchio and colleagues reported no toxic effects of the KU-60019 on the normal mouse brain, its effects in combination with radiation were not assessed (13). Secondly, the non-proliferative nature of the vast majority of normal brain cells indicates that they will be less susceptible to the effects of cell cycle checkpoint blockade, a theory that is supported by observations made by Moding and colleagues that ATM deletion *in vivo* does not affect the radiation sensitivity of non-proliferating normal tissues (14). Finally, studies on neural stem cell and progenitor populations in mice have shown that downregulation of ATM is associated with radioprotection, a somewhat surprising finding that has been attributed to ATM's pro-apoptotic role in these populations (15-17). Whether similar protective effects will be achieved with pharmacological inhibition of ATM, and in the context of clinical radiation schedules, is an important question that should inform the design and conduct of early phase clinical trials. In our opinion, these studies should be conducted in a cautious manner and should incorporate imaging and neurocognitive components to maximise information and minimise risks to the participating patients.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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