MET signaling promotes DNA repair and radiation resistance in glioblastoma stem-like cells

Pavlina Krasimirova Todorova, Bipasha Mukherjee, Sandeep Burma

Division of Molecular Radiation Biology, Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, TX, USA

Correspondence to: Sandeep Burma. Division of Molecular Radiation Biology, Department of Radiation Oncology, University of Texas Southwestern Medical Center, 2201 Inwood Road, NC7.208, Dallas, TX 75390, USA. Email: sandeep.burma@utsouthwestern.edu.

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Glioblastomas (GBMs) are primary brain tumors characterized by aggressive growth and rapid recurrence. They represent a leading cause of cancer-related deaths both in adult and pediatric patients (1,2). Despite the considerable success in understanding the genetic and molecular drivers of gliomagenesis, the current standard of care provides, at best, a transient remission. Newly-diagnosed GBM patients, who receive de-bulking surgery and adjuvant radiotherapy combined with temozolomide chemotherapy, have an average survival of 14.6 months (3). Invariably, patients succumb to recurrence-related death despite aggressive treatment regimens. The inevitable failure of current experimental and standard treatments has been attributed to two key features of GBM. First, the marked inter- and intra-tumoral heterogeneity prevents successful application of targeted monotherapies (4,5). Second, the existence of distinct GBM cancer stem cells which are refractory to treatment may drive tumor recurrence (5,6). These resilient stem-like cells present in the primary GBM are positively selected for, and oftentimes genetically altered, by ionizing radiation (IR) and/or temozolomide (7,8). As a result, the recurrent tumors no longer respond to radiation or chemotherapy. There is an urgent need to identify such therapy-resistant cells in the primary tumor, understand the molecular basis of therapy resistance, and develop strategies to effectively target these cells in primary or recurrent tumors.

Targeted IR obliterates cancer cells by inducing DNA double-strand breaks (DSBs) which trigger senescence or programmed cell death if left unrepaired (9). Therefore, in order for GBM stem-like cells to survive radiotherapy, they need to be better able to sense DSBs, initiate cell cycle checkpoints, and activate DSB repair mechanisms—processes which together constitute the so-called DNA damage response (DDR) (10). Indeed, several studies have reported increased activation of DDR pathways in radioresistant GBM cells (11-14). Ataxia telangiectasia mutated (ATM) is a central kinase that triggers the DDR response to DSBs (15). Hence, it is plausible that the heightened DDR capabilities of GBM stem-like cells might involve signaling pathways that impinge on ATM.

In a recent paper published in *EMBO Mol Med*, De Bacco and colleagues identify a signaling cascade triggered by the MET receptor tyrosine kinase (RTK) that leads to ATM hyper-activation in GBM stem-like cells, and provide mechanistic insights into the profound radioresistance of these cells (16). Physiologically, MET is important for epithelial-to-mesenchymal transition and cell migration during embryogenesis, tissue regeneration, and wound healing; in cancers, MET plays key roles in cell survival, invasion, and metastasis (17). Building on previous work from their and other laboratories showing that MET is a functional marker for a subset of GBM stem-like cells (18-20), in this study the authors examine whether MET expression might also distinguish a radioresistant subpopulation of GBM stem-like cells, and if MET

inhibition might help overcome radiation resistance in GBM (16).

De Bacco *et al.* have previously reported that about forty percent of neurosphere lines derived from primary GBM express MET (20). Such MET-positive neurospheres are a mix of cells that either highly express MET (MET^{high}) or that do not express MET at all (MET^{neg}); the MET^{high} subpopulation displays characteristics of cancer stem-like cells. In this study, they found that the MET^{high} subpopulation is selectively enriched upon irradiation of MET-positive neurophseres in culture (and also upon irradiation of tumors derived from these neurospheres). The authors hypothesized that the observed enrichment stems from an intrinsic radioresistance of the MET^{high} GBM stem-like cells which allows for their survival and expansion after IR both *in vitro* and *in vivo*. To test their hypothesis, the investigators sorted MET^{high} and MET^{neg} cells from MET-positive neurosphere lines, and found that the MET^{high} stem-like cells were indeed significantly more radioresistant than their MET^{neg} counterparts. Moreover, in the MET^{high} cells, the DDR kinase ATM and its downstream kinase CHK2 (15) were constitutively active, and their activities were further stimulated by IR. Such a heightened DDR response allowed these cells to efficiently repair radiation-induced DNA damage as evidenced by the faster resolution of IR-induced DNA breaks in the MET^{high} cells. The authors concluded that MET is a marker for a radioresistant GBM stem-like subpopulation, and that augmented DNA repair capabilities underlie the survival of these cells after IR.

De Bacco *et al.* demonstrated that addition of the MET-activating ligand, hepatocyte growth factor (HGF), enhanced survival of MET-positive neurospheres after radiation exposure, thereby confirming the role of MET signaling in radioresistance. Conversely, pre-treatment of these neurospheres with MET-specific inhibitors prior to IR significantly reduced viability. Importantly, the addition of HGF augmented radiation-induced ATM and CHK2 activation, while MET inhibitors attenuated these responses. To mechanistically elucidate the link between MET signaling and ATM activation, the authors examined two pathways downstream of MET, the PI3K-AKT and MAP kinase (MAPK) signaling cascades (17), and found that inhibition of PI3K, but not MAPK, decreased ATM and CHK2 phosphorylation. Moreover, they found that Aurora kinase A (Aurora-A) phosphorylation was stimulated by HGF, and suppressed by MET or PI3K inhibitors. Importantly, an Aurora-A inhibitor negatively affected IR-induced ATM phosphorylation and cell viability of MET-positive neurospheres, similar to that seen with MET inhibitors. Based upon these small molecule inhibitor studies, the authors postulate that a MET-AKT-Aurora-A pathway promotes ATM activation and radiation resistance in GBM stem-like cells.

AKT also appears to play an additional role in MET-mediated radioresistance. AKT-dependent phosphorylation of p21 has been shown to result in cytoplasmic retention of p21 where it exerts anti-apoptotic functions (21). De Bacco *et al.* show that MET activation in MET-positive neurospheres results in p21 phosphorylation and its cytoplasmic retention, and that this can be reversed by MET inhibition. Thus, MET appears to promote survival of GBM stem-like cells both by stimulating DSB repair as well as by inhibiting apoptosis.

Translationally, these results clearly indicate that MET inhibition may be a viable strategy for sensitizing GBM stem-like cells to IR in a tumor setting. To test this, the authors established tumors by intracranial or subcutaneous xenotransplantation of MET-positive neurosphere lines in mice. The tumors were treated with a combination of IR and JNJ38877605, a MET inhibitor that crosses the blood-brain barrier (22). They found that combination treatment delayed tumor growth significantly and increased survival of tumor-bearing mice (compared to radiation only). Importantly, combination treatment depleted the stem-like subpopulation within the treated tumors. Underscoring the clinical relevance of these findings, the authors also demonstrated enrichment for MET-positive cells post-IR in human GBM patients. They compared matched primary and recurrent (arising after surgery and adjuvant radio/chemotherapy) tumor samples. In the majority of cases, the recurrent tumors showed an increase in the number and staining intensity of MET-positive cells. If, as these results indicate, MET-positive cells indeed drive tumor recurrence in human patients, the use of MET inhibitors for treating the primary tumor might delay the onset of tumor recurrence.

In their paper, De Bacco *et al.*, provide a potential solution to a problem that has stalled the progress of GBM therapy for decades. By unraveling a signaling pathway important for radioresistance in patients expressing the MET RTK, they open new avenues for combined therapy. Inhibitors for MET, AKT, and Aurora-A are already in clinical trials, and most recently, crizotinib—a small-molecule MET inhibitor, has been shown to drive tumor regression in adult and pediatric glioma patients (2,23).

Although the effect of Crizotinib (or other inhibitors given as monotherapy) may be negated by the emergence of resistant clones, the authors' pre-clinical data provide a strong rationale for the concomitant administration of IR and MET inhibitors to achieve tumor remission. Importantly, the findings of De Bacco *et al.* may have broader implications for glioma therapy. Although a relatively small fraction of GBMs express MET, another RTK, EGFR, is amplified in approximately sixty percent of gliomas (24). Work done in our and other laboratories has shown that EGFR signaling can also modulate DNA repair and contribute to GBM radioresistance (13,25). Since EGFR signaling activates AKT (26), it would be of clinical interest to determine whether EGFR-driven radioresistance also operates through an AKT-Aurora-A-ATM phosphorylation cascade.

While this study illustrates how a subpopulation of MET-expressing stem-like cells in the primary tumor might survive radiation therapy and contribute to tumor recurrence (16), a previous study by the same group demonstrated that IR exposure also triggers transcriptional upregulation of MET in cancer cells (27). Of relevance to these findings, a study by our group involving mouse models of glioma showed that IR exposure commonly leads to genomic amplification of MET which drives gliomagenesis, and maintains a cancer stem-like phenotype in radiogenic gliomas (28). Taken together, these studies indicate that radiation therapy may not only select an existing subpopulation of MET-expressing cells in GBM, but might also engender new clones of tumor cells overexpressing MET. In this manner, IR could both generate and select MET-expressing tumor cells that would drive tumor recurrence and therapy resistance.

In sum, this paper reports a possible mechanism for GBM radioresistance and recurrence, and proposes a clinically feasible radiosensitization approach that targets GBM stem-like cells (16). This study also sets the stage for a number of basic biological questions that will hopefully be answered in the future. For example, how exactly does Aurora-A, a kinase known to be involved in the regulation of mitosis (29), augment ATM activation in response to IR? How does the MET-PI3K-AKT cascade regulate Aurora-A? Which DSB repair pathway(s) is specifically stimulated by the MET-Aurora-A-ATM axis in GBM stem-like cells? Clearly, there is still much to be learned about the role of MET in regulating DSB repair in cancer stem-like cells, and the study by De Bacco *et al.* (16) is a first step in that direction.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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