MOLECULAR & CELLULAR ONCOLOGY
2020, VOL. 7, NO. 6, e1834902 (3 pages)
https://doi.org/10.1080/23723556.2020.1834902

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COMMENTARY

Purine metabolism promotes radioresistance and is a therapeutic target in glioblastoma

Weihua Zhou and Daniel R Wahl

Department of Radiation Oncology, University of Michigan, Ann Arbor, MI, USA

ABSTRACT

Profound intratumoral genomic heterogeneity has limited the ability of targeted therapies to overcome therapy resistance in glioblastoma. We have defined purine metabolism as a key mediator of DNA repair and radiation resistance in glioblastoma. Because many glioblastoma oncogenic drivers activate purine metabolism, its inhibition may overcome therapy resistance despite intratumoral genomic heterogeneity.

ARTICLE HISTORY
Received 21 September 2020
Revised 2 October 2020
Accepted 6 October 2020

KEYWORDS
Glioblastoma; metabolism; purine synthesis; radiation; heterogeneity

TEXT

Glioblastoma (GBM) is the most prevalent and lethal primary invasive brain tumor in adults with a median survival of 1–2 years. Diverse molecular driver alterations have been defined in GBM. However, targeted therapies against these abnormalities have been ineffective, possibly due to the profound intratumoral genomic heterogeneity that characterizes GBMs[1,2] Consistent with this reasoning, the only therapies that have prolonged survival in GBM (surgery, radiation, temozolomide and tumor-treating fields) do not require a precise molecular alteration for efficacy.

Radiation (RT) is a critical treatment for nearly every patient with GBM. Despite its initial efficacy, more than 80% of GBMs recur within the high dose RT field. Thus, overcoming RT-resistance is likely to extend the survival of GBM patients. Because of the limitations of targeted therapies in the face of molecularly heterogeneous tumors, our group set out to define common mediators of GBM RT-resistance that might exist across varied oncogenic drivers.

Altered metabolism is a hallmark of cancers including GBM, and could potentially regulate therapy resistance independently of genotype. Indeed, different oncogenic alterations can activate common metabolic pathways.[3] We therefore began investigating how metabolism mediates RT-resistance in GBM.

We first performed clonogenic survival assays on 23 GBM cell lines and found a wide distribution of intrinsic RT sensitivities. We then performed targeted metabolomic analysis on these same cell lines during unperturbed exponential growth. We correlated metabolite abundance with RT-resistance across all cell lines and found that accumulation of metabolites involved in *de novo* purine synthesis (inosinates and guanylates) was correlated with RT-resistance. We then treated GBM cells with RT and assessed their metabolism 2 h later. RT-resistant GBMs were able to acutely increase both pyrimidines and purines, while RT-sensitive GBMs were not. Guanylates remained the metabolic pathway most associated with GBM RT-resistance following RT.

To determine whether this correlation between nucleobase-containing metabolites and GBM RT-resistance was causal, we perturbed purine and pyrimidine pools in numerous ways. Supplementing RT-sensitive GBM cell lines and neurospheres with combined purines and pyrimidines protected these cells from RT by speeding the repair of RT-induced DNA double strand breaks (DSBs).

We then depleted nucleotide pools in RT-resistant GBM models to attempt to overcome RT-resistance. We began with mycophenolic acid (MPA), a Food and Drug Administration (FDA)-approved inhibitor of Guanosine-5′-triphosphate (GTP) synthesis. MPA depleted GBM GTP concentrations and sensitized multiple GBM cell lines and neurospheres to RT in a concentration-dependent fashion. MPA also slowed the ability of these cells to repair RT-induced DSBs. These beneficial effects of MPA were reversed by nucleoside supplementation indicating that there were due to the ability of the drug to deplete GTP rather than off-target effects.

MPA inhibits inosine-5′-monophosphate dehydrogenase (IMPDH), which catalyzes the *de novo* synthesis of GTP from individual amino acids, glucose-derived ribose and one carbon units. IMPDH is also used by an arm of the GTP salvage pathway, when the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT) joins the base hypoxanthine to phosphoribosyl pyrophosphate to form inosine monophosphate (IMP), which is then metabolized by IMPDH to eventually form GTP. Thus, it was not clear whether MPA treatment overcame GBM RT-resistance by inhibiting *de novo* or salvage GTP synthesis. However, inhibiting IMPDH-dependent GTP salvage by restricting hypoxanthine or silencing the gene encoding HGPRT did not affect RT-resistance, suggesting that *de novo* GTP synthesis is critical for GBM RT-resistance.

CONTACT Daniel R Wahl dwahl@med.umich.edu University of MichiganAnn Arbor, MI 48109, USA

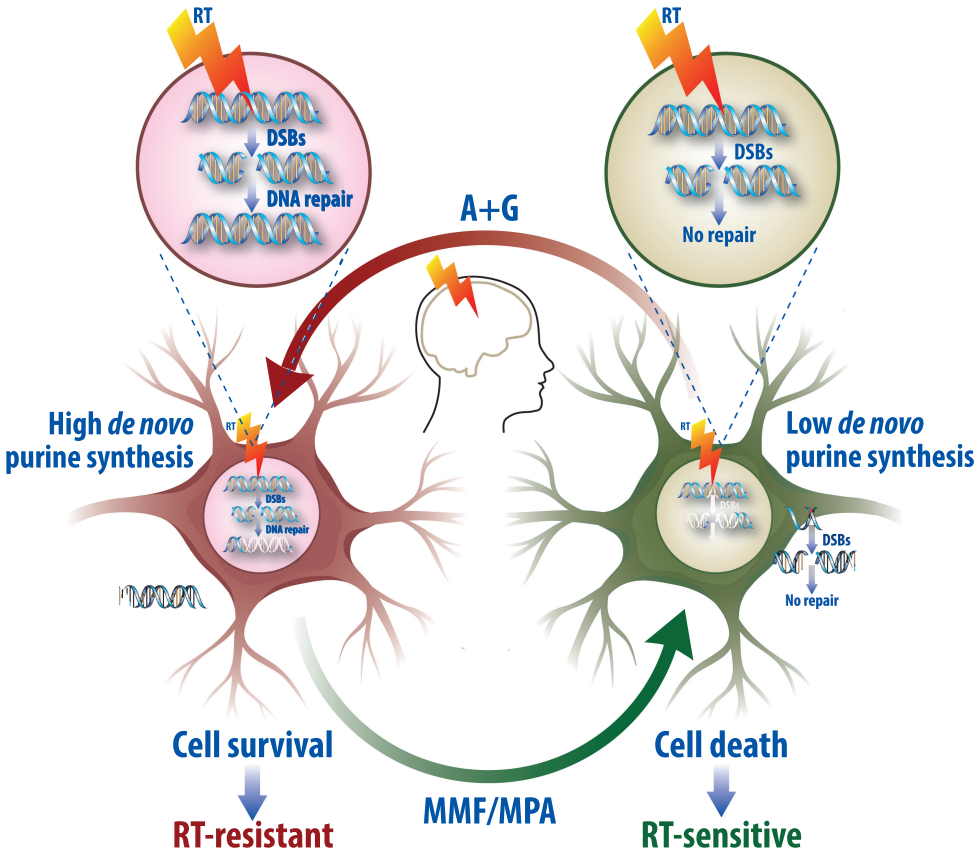

Figure 1. Elevated purine synthesis favors radioresistance in glioblastoma, which can be reversed by MPA/MMF. High *de novo* purine synthesis stimulates double-strand DNA break repair, promotes cell survival and induces recurrence after RT in GBMs. Supplementing cells with purines (A + G) promotes RT-resistance while inhibition of *de novo* purine synthesis with MPA/MMF promotes RT-sensitivity. Note: RT: radiation; MPA: mycophenolic acid; MMF: mycophenolate mofetil; A: Adenosine; G: Guanosine.

We then asked whether pyrimidines played a similar role. We treated RT-sensitive GBM cells separately with either purines or pyrimidines, and found that purines alone conferred protection from RT. We then depleted pyrimidine pools using teriflunomide, an FDA-approved inhibitor of *de novo* pyrimidine synthesis. Pyrimidine depletion neither radiosensitized GBM models nor affected their ability to repair RT-induced DSBs.

We next sought to confirm these findings *in vivo*. We utilized mycophenolate mofetil (MMF), which is an orally bioavailable pro-drug of MPA that is FDA approved to treat organ rejection, in flank models of GBM. We confirmed that MMF, when given in combination with RT, suppressed tumor growth and extended mouse survival by depleting intratumoral guanylate levels. Since the GBM microenvironment and poor intracranial exposure can affect drug efficacy, we then asked whether MMF would be effective in an intracranial GBM model. We found that combined MMF and RT prolonged mouse survival in a RT-resistant orthotopic patient-derived-xenografts (PDX) model of primary GBM. Hence, we found that inhibition of GTP synthesis with MMF can intracranially overcome RT-resistance of GBM.

Together, our findings indicate that purine metabolism, especially the *de novo* synthesis of GTP, is a key mediator of DNA repair and RT-resistance in GBM[4] (Figure 1). Importantly, many of the heterogeneous oncogenic alterations that drive GBM promote *de novo* purine synthesis .[5,6] Thus, a molecularly heterogeneous GBM may exhibit a relatively homogeneous elevation of *de novo* purine synthesis, which could be exploited therapeutically with MMF to overcome RT-resistance.

Our findings have motivated the development of a clinical trial to evaluate the effects of MMF for a radiosensitizer to treat GBM patients (NCT04477200). In this study, we aim to find the maximum safe dose of MMF that can be combined with RT and make a preliminary estimate of whether that MMF at that dose is efficacious. Further, by analyzing tumor tissue in patients receiving MMF who are undergoing resection, we will confirm that MMF achieves active concentrations intracranially. Because normal brain tissues including neural stem cells preferentially rely on purine salvage, we are optimistic that this strategy will have minimal normal tissue toxicity. We are hopeful that targeting GBM metabolism may improve the dismal outcomes currently expected for patients with this disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

The work was supported by grants from the American Cancer Society, the Forbes Institute for Cancer Discovery, the National Cancer Institute (K08CA234416) and the Jones Family Foundation Fund within the Chad Carr Pediatric Brain Tumor Center to D.R.W. and by the Postdoctoral Translational Scholar Program (UL1TR002240) from the Michigan Institute for Clinical & Health Research of University of Michigan to W.Z.

ORCID

Weihua Zhou http://orcid.org/0000-0003-0259-0828
Daniel R Wahl http://orcid.org/0000-0001-9894-5085

References

1. Raizer JJ, Abrey LE, Lassman AB, Chang SM, Lamborn KR, Kuhn JG, Yung WKA, Gilbert MR, Aldape KA, Wen PY, et al. A phase II trial of erlotinib in patients with recurrent malignant gliomas and nonprogressive glioblastoma multiforme postradiation therapy. Neuro Oncol. 2010 Jan;12(1):95–103. doi:10.1093/neuonc/nop015.
2. Pitz MW, Eisenhauer EA, MacNeil MV, Thiessen B, Easaw JC, Macdonald DR, Eisenstat DD, Kakumanu AS, Salim M, Chalchal H, et al. Phase II study of PX-866 in recurrent glioblastoma. Neuro Oncol. 2015Sep, 17(9), 1270–1274.
3. Krishnankutty R, Iskandarani A, Therachiyil L, Uddin S, Azizi F, Kulinski M, Bhat AA, Mohammad RM. Anticancer activity of camel milk via induction of autophagic death in human colorectal and breast cancer cells. Asian Pac J Cancer Prev. 2018;19(12):3501–3509. doi:10.31557/APJCP.2018.19.12.3501. Roopesh Krishnankutty et al. Asian Pac J Cancer Prev. 2020 May 1;21(5):1495
4. Zhou W, Yao Y, Scott AJ, Wilder-Romans K, Dresser JJ, Werner CK, Sun H, Pratt D, Sajjakulnukit P, Zhao SG, et al. Purine metabolism regulates DNA repair and therapy resistance in glioblastoma. Nat Commun. 2020 Jul 30;11(1):3811. doi:10.1038/s41467-020-17512-x.
5. Saha A, Connelly S, Jiang J, Zhuang S, Amador D, Phan T, Pilz R, Boss G. Akt phosphorylation and regulation of transketolase is a nodal point for amino acid control of purine synthesis. Mol Cell. 2014 Jul 17;55(2):264–276. doi:10.1016/j.molcel.2014.05.028.
6. Jin N, Bi A, Lan X, Xu J, Wang X, Liu Y, Wang T, Tang S, Zeng H, Chen Z, et al. Identification of metabolic vulnerabilities of receptor tyrosine kinases-driven cancer. Nat Commun. 2019 Jun 20;10(1):2701. doi:10.1038/s41467-019-10427-2.