Editorial

Glioblastoma radiosensitization by pimozide

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Comment on: Lee JK, Chang N, Yoon Y, *et al.* USP1 targeting impedes GBM growth by inhibiting stem cell maintenance and radioresistance. Neuro Oncol 2016;18:37-47.

Submitted Sep 19, 2016. Accepted for publication Oct 09, 2016.

doi: 10.21037/tcr.2016.11.17

View this article at: http://dx.doi.org/10.21037/tcr.2016.11.17

Glioblastoma (GBM) is the most common form of malignant brain tumor corresponding to approximately 20,000 new cases in the US per year (1). While the best available treatment involves surgery followed by the combination of radiotherapy and temozolomide (2), the survival of the vast majority of patients with GBM remains less than 2 years after diagnosis. Accordingly, considerable research has continued into the development of more effective GBM therapy. At the fundamental and preclinical levels such research has focused on developing experimental models that accurately reflect GBM biology, defining the molecules mediating GBM cell survival and treatment resistance and identifying drugs suitable for targeting those molecules within the constraints of CNS physiology. In the recent article by Lee *et al.* (3), each of these topics has been addressed in an attempt to suggest a novel, more effective GBM treatment strategy.

With respect to an experimental model, laboratory investigations of GBM often employ long established glioma cell lines, which in terms of genetic abnormalities, gene expression profiles and orthotopic growth patterns, have little in common with GBMs in situ (4). With respect to a more biologically accurate model system, data now suggest that GBMs are driven and maintained by a subpopulation of clonogenic cells referred to as glioblastoma stem-like cells (GSCs). Isolation of GSCs entails generation of neurosphere cultures from human GBM surgical specimens; a critical aspect of this process is the use of defined, serum-free media (5). In contrast to the traditional glioma cell lines, GSCs simulate the genotype and gene expression patterns of the GBM from which they originated and when grown orthotopically in immuno-compromised mice they grow as invasive neoplasms comprised of heterogeneous subpopulations (6). Finally, brain tumor xenografts initiated from GSCs appear to replicate the GBM radioresistance observed clinically (7). In their paper published in *Neuro-Oncology*, Lee *et al.* used the GSC model to test the role of the deubiquitinating (DUB) enzyme USP1 in mediating survival and radioresistance of GBM cells and then to serve as a target for brain tumor therapy.

As recently reviewed by Kee and Huang (8), there are about 95 DUBs that can be divided into 5 categories, one of which is ubiquitin-specific proteases (USPs). Given their roles in cancer-related signaling and DNA repair (8,9), DUBs in general have been suggested as therapeutic targets. Along these lines, USP1 has been shown to stabilize the transcription factor ID1 (10), which is overexpressed in GBMs and has been linked to the stem-like phenotype of GSCs (11). Lee *et al.* show that knockdown of USP1 or inhibition of its activity leads to a reduction in ID1 levels along with the reduced expression of ID1 regulated stem cell associated genes *SOX2* and *OLIG2*. These molecular effects were then associated with a decrease in GSC proliferation and survival as well as a reduction in the growth of orthotopic xenografts initiated from GSCs. Thus, as previously reported for other tumor cell types (10,12), targeting USP1 as a means of reducing the levels of the critical tumor stem cell transcription factor ID1 appears to be a potential GBM treatment strategy.

However, the concept of targeting USP1 as an approach to enhancing GBM radiosensitivity appears to be somewhat tenuous. Whereas DUBs as well as a number USPs contribute to the repair of DNA double strand breaks (13), the critical lesion responsible for radiation-

induced cell death, USP1 has been primarily implicated in the replication-associated DNA damage response, which mediates the repair of interstrand crosslinks and translesion synthesis, DNA damage not traditionally linked to cell death after radiation exposure. Knockout of the *USP1* gene in DT40 chicken cells (14) has no effect on in vitro radiosensitivity and in mouse embryonic fibroblasts has only a minor effect (15). In the analyses of GSC radiosensitivity by Lee *et al*., no data were presented using an approach to specifically target USP1 (i.e., shRNA) as was applied in the initial experiments in their manuscript evaluating gene expression and overall cell survival. The radiosensitization experiments only involved the use of pimozide, which in addition to inhibiting USP1 (16) targets a number of other molecules and processes such as STAT3 (17), cholesterol synthesis (18), sigma receptors (19) and wnt/β-catenin (20): all of which have been implicated as targets for radiosensitization. Although pimozide has previously been reported to inhibit USP1 and to reduce ID1 levels (12), in Lee *et al*., the concentration of pimozide shown to reduce ID1 levels in GSCs was five times that used in the clonogenic analysis of radiosensitization. Thus, although pimozide enhanced GSC radiosensitivity, whether USP1 is the target remains to be specifically determined. With respect to GBM therapy, given the role of USP1 in translesion repair synthesis, it would be of interest to determine the effects of USP1 knockdown on GSC sensitivity to the alkylating agent temozolomide.

Clonogenic analysis clearly indicates that pimozide enhances the in vitro radiosensitivity of a GSC line, which is consistent with a previous report using the breast carcinoma cell line MCF7 (19). Importantly, for mice bearing orthotopic xenografts initiated from a different GSC line the combination of pimozide and fractionated irradiation resulted in a substantially greater than additive increase in survival compared to either treatment alone. Whereas the mechanisms mediating pimozide-induced radiosensitization including a role of USP1 remain to be defined, pimozide does appear to have significant radiosensitizing potential applicable to GBM. Towards this end, pimozide has a long history of use in the treatment of schizophrenia and Tourette's syndrome, which has been attributed to blocking postsynaptic dopaminergic receptors (21). A major impediment in the development of effective chemotherapy for GBM as compared to other tumor sites is the blood-brain barrier (BBB), which limits the transport of many of the standard cytotoxic drugs (e.g., cisplatin) as well as targeted agents (e.g., Herceptin) into the CNS and thus restricting tumor exposure to potentially effective drugs. An approach to overcoming the BBB is to "repurpose" the multitude of drugs already in use to treat neurological diseases (22). These agents not only cross the BBB but there is usually considerable information available regarding their pharmacokinetics (PK) and toxicity profiles. An example of repurposing drugs for GBM therapy is the use of valproic acid (VPA), a long used anti-seizure medication, as a novel radiosensitizer. Though the specific molecular processes mediating VPA's anti-seizure effects are not completely understood, among its established activities is the inhibition of histone deacetylase activity (23), a process shown to enhance tumor cell radiosensitivity (24). A recently completed phase 2 trial of VPA added to the combination of temozolomide and radiotherapy in patients with newly diagnosed GBM showed an extension of the median survival from 14.2 to 29.6 months (25). The overall side effect profile was very low and not outside that expected for patients treated with VPA or radiotherapy/temozolomide. Because of the long experience using VPA the investigators could prepare for the drug's known toxicities thus making the combination trial with two other agents safer. For example, TMZ is known to cause thrombocytopenia as can VPA prompting the weekly evaluation of platelet levels as a precaution instead of typical monthly lab draws. As such there were no grade 4 thrombocytopenia events in this trial during the VPA/RT/TMZ treatment.

While additional preclinical investigations are required, the article by Lee *et al*. supports the further evaluation of pimozide as an addition to the current standard of care for GBM. From a larger perspective this paper demonstrates some of the benefits and problems associated with repurposing drugs that are already FDA approved. On the positive side the drugs are available, packaged and ready to be given to patients. Information about PK, pharmacodynamics (PD) and toxicity will have already been published. Additionally, depending on the age of the drug it may be fairly inexpensive, especially if there is a generic form. On the complicating side the PK, PD and toxicology may be for a very different dose of drug and will not likely be in combination with anti-cancer agents. Similarly, the PD would likely be for a different target. For example, in the Lee paper pimozide was developed to target postsynaptic dopaminergic receptors, which is not the likely target as a radiation sensitizer. An additional complicating factor is that the drug may be off patent or the pharmaceutical company may not be experienced nor interested in pursuing oncology studies. However, Lee *et al*. have demonstrated that "old" drugs may be used for new purposes.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Hongcheng Zhu (Department of Radiation Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2016.11.17). The authors have no conflicts of interest to declare.

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.

References

1. CBTRotUS. Central Brain Tumor Registry of the United States. Available online: http://www.CBTRUS.org
2. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-96.
3. Lee JK, Chang N, Yoon Y, et al. USP1 targeting impedes GBM growth by inhibiting stem cell maintenance and radioresistance. Neuro Oncol 2016;18:37-47.
4. Li A, Walling J, Kotliarov Y, et al. Genomic changes and gene expression profiles reveal that established glioma cell lines are poorly representative of primary human gliomas. Mol Cancer Res 2008;6:21-30.
5. Lee J, Kotliarova S, Kotliarov Y, et al. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 2006;9:391-403.
6. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003;63:5821-8.
7. Jamal M, Rath BH, Williams ES, et al. Microenvironmental regulation of glioblastoma radioresponse. Clin Cancer Res 2010;16:6049-59.
8. Kee Y, Huang TT. Role of Deubiquitinating Enzymes in DNA Repair. Mol Cell Biol 2015;36:524-44.
9. García-Santisteban I, Peters GJ, Giovannetti E, et al. USP1 deubiquitinase: cellular functions, regulatory mechanisms and emerging potential as target in cancer therapy. Mol Cancer 2013;12:91.
10. Williams SA, Maecker HL, French DM, et al. USP1 deubiquitinates ID proteins to preserve a mesenchymal stem cell program in osteosarcoma. Cell 2011;146:918-30.
11. Soroceanu L, Murase R, Limbad C, et al. Id-1 is a key transcriptional regulator of glioblastoma aggressiveness and a novel therapeutic target. Cancer Res 2013;73:1559-69.
12. Mistry H, Hsieh G, Buhrlage SJ, et al. Small-molecule inhibitors of USP1 target ID1 degradation in leukemic cells. Mol Cancer Ther 2013;12:2651-62.
13. Jackson SP, Durocher D. Regulation of DNA damage responses by ubiquitin and SUMO. Mol Cell 2013;49:795-807.
14. Oestergaard VH, Langevin F, Kuiken HJ, et al. Deubiquitination of FANCD2 is required for DNA crosslink repair. Mol Cell 2007;28:798-809.
15. Kim JM, Parmar K, Huang M, et al. Inactivation of murine Usp1 results in genomic instability and a Fanconi anemia phenotype. Dev Cell 2009;16:314-20.
16. Chen J, Dexheimer TS, Ai Y, et al. Selective and cell-active inhibitors of the USP1/ UAF1 deubiquitinase complex reverse cisplatin resistance in non-small cell lung cancer cells. Chem Biol 2011;18:1390-400.
17. Chen JJ, Cai N, Chen GZ, et al. The neuroleptic drug pimozide inhibits stem-like cell maintenance and tumorigenicity in hepatocellular carcinoma. Oncotarget 2015. [Epub ahead of print]
18. Wiklund ED, Catts VS, Catts SV, et al. Cytotoxic effects of antipsychotic drugs implicate cholesterol homeostasis as a novel chemotherapeutic target. Int J Cancer 2010;126:28-40.
19. Strobl JS, Melkoumian Z, Peterson VA, et al. The cell death response to gamma-radiation in MCF-7 cells is enhanced by a neuroleptic drug, pimozide. Breast Cancer Res Treat 1998;51:83-95.
20. Fako V, Yu Z, Henrich CJ, et al. Inhibition of wnt/

β-catenin Signaling in Hepatocellular Carcinoma by an Antipsychotic Drug Pimozide. Int J Biol Sci 2016;12:768-75.
21. Schwinn G, Schwarck H, McIntosh C, et al. Effect of the dopamine receptor blocking agent pimozide on the growth hormone response to arginine and exercise and on the spontaneous growth hormone fluctuations. J Clin Endocrinol Metab 1976;43:1183-5.
22. Lee JK, Nam DH, Lee J. Repurposing antipsychotics as glioblastoma therapeutics: Potentials and challenges. Oncol Lett 2016;11:1281-6.
23. Göttlicher M, Minucci S, Zhu P, et al. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J 2001;20:6969-78.
24. Camphausen K, Cerna D, Scott T, et al. Enhancement of in vitro and in vivo tumor cell radiosensitivity by valproic acid. Int J Cancer 2005;114:380-6.
25. Krauze AV, Myrehaug SD, Chang MG, et al. A Phase 2 Study of Concurrent Radiation Therapy, Temozolomide, and the Histone Deacetylase Inhibitor Valproic Acid for Patients With Glioblastoma. Int J Radiat Oncol Biol Phys 2015;92:986-92.

Cite this article as: Rath BH, Camphausen K, Tofilon PJ. Glioblastoma radiosensitization by pimozide. Transl Cancer Res 2016;5(Suppl 6):S1029-S1032. doi: 10.21037/tcr.2016.11.17

