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Glioblastoma radiosensitization by pimozone

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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

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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 of 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 pimozone, 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 pimozone has previously been reported to inhibit USP1 and to reduce ID1 levels (12), in Lee *et al.*, the concentration of pimozone shown to reduce ID1 levels in GSCs was five times that used in the clonogenic analysis of radiosensitization. Thus, although pimozone 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 pimozone 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 pimozone and fractionated irradiation resulted in a substantially greater than additive increase in survival compared to either treatment alone. Whereas the mechanisms mediating pimozone-induced radiosensitization including a role of USP1 remain to be defined, pimozone does appear to have significant radiosensitizing potential applicable to GBM. Towards this end, pimozone 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 pimozone 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 pimozone 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.

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