Capitalizing on ATRX loss in glioma via PARP inhibition: Comment on “Loss of ATRX confers DNA repair defects and PARP inhibitor sensitivity” by Garbarino et al.

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Gliomas are devastating tumors of the central nervous system for which there is a dire need to expand current treatment options. The greatest strides to this end will come from a deeper understanding of key mechanisms that gliomas depend on for continued growth. Garbarino et al. do just that, and not only provide insight into DNA repair mechanisms in glioma, but they also discover a potential drug combination to use in certain gliomas that can robustly increase tumor cell death. In particular, the authors elucidate the role of Alpha thalassemia/mental retardation syndrome X-Linked (ATRX) in glioma and in doing so, provide insight as to how ATRX functions in glioma and how it can be used as a therapeutic target (Fig. 1).

ATRX is a protein most well-known and understood for its role as a histone chaperone that aids in incorporating histone H3.3 into the genome. It has been implicated in DNA damage response (DDR) pathways, but its full function is not entirely understood [1]. It has been shown that mutations in ATRX can lead to genomic instability, defects in nonhomologous end joining, and an increase in activity of the alternative lengthening of telomeres (ALT) pathway. The correlation between ATRX and ALT activity in tumors highlights the need to further elucidate potential additional functions in cancer [2–4]. Mutations in ATRX are seen in gliomas and occur along with Isocitrate dehydrogenase (IDH) mutations. IDH mutations occur in the vast majority of lower grade glioma (>80%), and predict better survival outcomes [5]. Similarly, ATRX mutations are fairly common in low grade glioma, but relatively rare in higher grade glioma. Despite the fact that ATRX mutations are frequently documented in glioma, and even used as diagnostic criteria, the cellular impact of the mutations is still not fully understood nor is the potential to therapeutically exploit tumors with mutant ATRX.

To understand the goal of this study, it is important to note that IDH mutant tumors have homologous recombination (HR) defects, which confer Poly(ADP)-ribose polymerase (PARP) inhibitor sensitivity [6]. PARP is an enzyme involved in the repair of single-strand DNA breaks. In tumors with HR defects, PARP inhibitors (PARPi) have been effective in decreasing tumor progression; the classic example of this is in breast and ovarian cancer with BRCA mutations [7]. In this study, the authors hypothesized that due to the fact that ATRX is likely involved in the DDR, ATRX mutations would confer PARPi sensitivity in glioma cells. Furthermore, they posited that ATRX mutations in cells that also contain IDH mutations would have increased sensitivity to PARPi over either mutation alone.

To begin testing their hypothesis, the authors generated ATRX knockout (KO) cells using CRISPR/Cas9 gene editing in immortalized astrocytes and confirmed functional loss of ATRX by multiple methods. After generation of the KO cell line, a drug screen that focused on DNA damaging agents and repair inhibitors was performed on wild type control cells and KO cells. This screen was used to identify which drugs were specifically effective at increasing cell death in cells with ATRX loss. From this screen, the authors noted significant sensitivity of the ATRX KO cells to two different PARPi, olaparib and talazoparib. The authors then went on to show that both drugs had a synthetic lethal interaction with the loss of ATRX, both by a short term cell viability assay, and by a clonogenic survival assay. In addition to demonstrating this effect in their CRISPR KO cells, they also showed the same effect in a glioma cell line expressing an inducible short hairpin RNA (shRNA) to ATRX.

After establishing that loss of ATRX confers sensitivity to PARPi, the authors then began to explore how this loss impacted DNA damage and repair. They hypothesized that ATRX loss led to replication stress, and confirmed this by the presence of the replication stress marker, phosphorylated Replication protein A (pRPA), with the ATRX mutation alone. In addition, they found further elevation of pRPA and activation of another key player in the response to replication stress, Checkpoint...
kinase 1 (Chk1), in response to olaparib treatment. This demonstrated that the loss of ATRX allows some amount of replication stress, but this is exacerbated to the point of activating the Ataxia telangiectasia and Rad3-related (ATR) replication stress signaling axis when olaparib is present.

Importantly, an increase in PARPi sensitivity has been reported in IDH mutant tumors [6]. Considering ATRX loss almost always occurs with IDH mutations in glioma, the authors then determined if having both of these mutations increased PARPi sensitivity even further. To test this, olaparib was administered to wild type and KO cells that contained an inducible IDH R132H mutation. The combined loss of ATRX and the IDH mutation did not lead to an increase in sensitivity over either mutation alone. This was also confirmed by administering olaparib to cells containing inducible shRNA to both ATRX and IDH1; this model also showed no increased sensitivity to PARPi when both mutations are present. Lastly, it has been previously demonstrated that the combination of ATR inhibitors (ATRi) and PARPi have a synergistic effect on HR-defective tumors or tumors with replication stress [8]. Considering this, and the fact that ATRX KO cells have activated ATR signaling, the authors next tested whether this synergy could be observed in their models as well. Indeed, the loss of ATRX conferred increased sensitivity to the combination of ATRi and PARPi.

PARPi inhibitors have been clinically investigated for over 15 years, but only more recently have been used in the context of glioma. In fact, PARPi inhibitors are currently in Phase I and Phase II clinical trials for patients with recurrent IDH-mutant glioma. As mentioned earlier, loss of ATRX in glioma rarely, if ever, occur without an IDH mutation [9]. This fact, combined with the results of this current study, provide even more rationale to use PARPi in patients with IDH, especially those with ATRX loss. It would seem, from the results of this study, that the combination of IDH mutation and ATRX does not confer any extra sensitivity to PARPi, though this is absolutely an area that could warrant further investigation. This study also looks at the combination of PARPi and ATRi, considering the finding that ATRX loss activates the ATR signaling axis. It is already known that this combination is synergistic in the context of IDH mutant tumors, and the authors find that this is true for ATRX KO cells as well, providing excellent rationale for clinical investigation of this drug combination in patients with IDH mutant/ATRX mutant gliomas.

Not only do the authors demonstrate findings that are promising for future clinical studies, but they also further understanding of the role of ATRX in the DDR, and what happens when it is mutated in gliomas. The authors mention early on that the role of ATRX in the DDR is not fully known, and prior investigations have produced conflicting results. However, in this study, they demonstrate that ATRX loss increased activation of the ATR signaling axis. This association between ATRX loss and HR defects/replication stress is further bolstered by the fact that ATRX loss confers PARPi sensitivity. Clinical reports of ATRX loss in glioma would suggest that ATRX mutations without IDH are rare, but this study shows that if this is the case in a patient glioma, PARPi inhibitors and/or ATR inhibitors are still a very good option for treatment. Additionally, it provides further justification for the use of PARPi in IDH mutant tumors, something that is already being actively investigated. Lastly, ATRX mutations are not only found in glioma, and can be identified in osteosarcoma, adrenocortical carcinoma, pancreatic neuroendocrine tumors, and other cancers [1]. This study provides rationale for the use of PARPi and ATR in these tumors as well, even if they do not carry IDH mutations. This study holds widespread implications not only for advancing our understanding of ATRX mutations in glioma, but also for clinical management of glioma, and potentially even other cancers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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