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Targeting pyrimidine metabolism for glioblastoma therapy

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See the article by Echizenya and Ishii et al. in this issue, pp. 229–239.

Glioblastoma (GBM) is the most aggressive and common brain tumor in adults. Despite aggressive surgical resection followed by concomitant administration of temozolomide (TMZ) and ionizing radiation, these tumors relapse and lead to a poor prognosis in affected patients. Molecular and cellular deconvolution of brain tumors at single cell resolution is rapidly evolving, but translating this knowledge to new therapies is progressing at a slower pace than desired.[1] In this issue, Echizenya et al[2] demonstrate that a chemical compound library developed by Fujifilm can be repurposed to identify novel targets that sensitize glioma-initiating cells (GICs) to TMZ. In their primary screen, the authors selected 319 compounds from a 10 560 compound library based on 20% cell viability in TMZ-resistant glioma stem cells (GSCs). In a subsequent secondary screening by using 2 independent TMZ-resistant and -sensitive GSCs, they narrowed the list to 302 compounds. Importantly, by including normal astrocytes and neural stem cells in their third screen, the authors filtered out potential toxic compounds that could affect healthy cells. This yielded 12 compounds, out of which they selected 7 compounds that had a 50% growth inhibition at less than 1 μM. Finally, the authors distilled to 2 compounds, 9700 and 10607, based on their structural similarity and strong antiproliferative properties against GICs.

Although cytotoxic, the precise targets of these compounds remained unknown. To address this, the authors constructed probe conjugates using a FLAG tag and analyzed the peptides using proteomics based on liquid chromatography–tandem mass spectrometry and compared them with control probes. From these analyses, they identified dihydroorotate dehydrogenase (DHODH), an enzyme involved in de novo pyrimidine biosynthesis, as a potential target of the hit compounds and confirmed it by in vitro enzyme inhibition assay. To further strengthen the observation that DHODH could be a viable cancer target, the authors used a focused screen combined with a pharmacokinetic screen for DHODH inhibitors in the Fujifilm compound library and found a highly stable DHODH inhibitor, 10580.

DHODH catalyzes the conversion of dihydroorotate to orotate (orotic acid), an essential step in the generation of uridine monophosphate, which is essential for the generation of pyrimidine.[3] Metabolic adaptation is one of the vital hallmarks of cancer, and rapidly proliferating cells depend on de novo nucleotide biosynthesis; enzymes that are involved in de novo biosynthesis such as DHODH are overexpressed in cancer.[3] The pyrimidine metabolic signature results in poor clinical outcomes in gliomas.[4] Therefore, inhibition of DHODH is an attractive target for cancer therapy.[3] It is interesting to note that DHODH is overexpressed in high-grade gliomas and in GICs compared with matched differentiated GICs.[4] DHODH inhibition induces cell differentiation in neural crest stem cells and in acute myeloid leukemia cells in addition to inhibiting cellular growth.[5,6] Although the compounds identified in this study decrease stemness by inhibiting translocation of sex determining region Y–box 2 (Sox2), a key stem cell maintenance factor, to the nucleus, an additional series of experiments by the authors identified that the O-GlcNAcylation, a posttranslation modification that involves the addition of O-linked N-acetylglucoamine (o-GlcAc), is essential for Sox2 nuclear translocation and induction of proliferation. Interestingly, DHODH inhibition significantly decreased the O-GlcNAcylation substrate uridinediphosphate N-acetylglucosamine (UDP-GlcNAc) and thereby preventing the Sox2 nuclear translocation in GICs via inhibiting Sox2 binding to exportin 1 (also known as CRM1 [**chromosomal maintenance 1**]).

Owing to the 10580 compound's poor penetrability of the blood–brain barrier (BBB), the authors opted for an in vivo subcutaneous GBM mouse model and demonstrated decreases in tumor volume. Despite the higher cytotoxicity of 10580 compared with the FDA-approved pyrimidine inhibitors teriflunomide and leflunomide, poor BBB penetrability limits

its translational utility for brain tumors. A recent study, however, supports the notion of exploring DHODH-based therapies for GBM wherein combining the DHODH inhibitor teriflunomide with the epidermal growth factor receptor inhibitor lapatinib or the phosphatidylinositol-3 kinase inhibitor BKM120 significantly improved survival in an orthotropic intracranial GBM mouse model.[4] The one potential caveat of DHODH inhibitors is that they cannot be combined with immune checkpoint inhibitors, since the DHODH inhibition induces immunosuppression.[7,8] Although DHODH inhibitors and their relation to cellular differentiation are not well studied, transcriptional elongation has been proposed as a possible mechanism in melanoma.[5] Indeed, new-generation DHODH inhibitors are in phases I and II clinical trials as differentiation therapies in AML.[3] It will be very interesting to see the DHODH inhibitor's effect on the differentiation of GSCs. Cells deficient in phosphatase and tensin homolog (PTEN) have been reported to be particularly sensitive to DHODH inhibition due to their augmented glutamine metabolism.[9] Since PTEN deletion/mutation is a common event in GBM, it will be interesting to determine the relationship of genomic aberrations in PTEN and DHODH dependency. The current study by Echizenya et al is unique in several ways. First, the authors used an unconventional chemical compound library to screen and repurpose the compounds indented for the photography and printing industry. Second, they examined efficacy of the compounds in normal neural stem cells and astrocytes to filter out the potential compounds that could be toxic to normal cells. Third, proteomic approaches identified DHODH as a target in an unbiased manner, which is essential when repurposing chemical compounds that are not designated for medicinal purposes. Finally, the authors incorporated in vitro pharmacokinetic approaches to select for potential compounds that could have translatable potential. Overall, this study opens up the possibility of targeting key metabolic vulnerabilities such as pyrimidine synthesis for the treatment of brain tumors.

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