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# Targeting nicotinamide adenosine dinucleotide (NAD) in diffuse gliomas

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Diffuse gliomas are the most common primary brain tumors and are extremely difficult to treat, regardless if they are lower grade gliomas or aggressive glioblastomas at initial diagnosis. Due to the high level of intra- and inter-tumoral heterogeneity and a genomic landscape that constantly evolves due to selective pressure in response to the therapies, treatments that target individual signal transduction pathways in gliomas have failed to improve clinical outcomes. Despite their genetic heterogeneity, all cancer cells need to reprogram metabolic pathways to balance the need for biosynthesis of cell building blocks and sufficient ATP to support cell growth and survival. Metabolic reprogramming is considered a hallmark of cancer.[1]Therefore, there is a growing interest in developing strategies to target tumor-specific metabolic processes that are critical for cancers, including gliomas.

Nicotinamide adenosine dinucleotide (NAD) has emerged as one of the most important factors involved in both bioenergetic and regulatory processes.[2] NAD is an essential electron carrier in redox reactions involved in a number of metabolic pathways such as glycolysis, oxidative phosphorylation, and the tricarboxylic acid (TCA) cycle.[3] An elevated level of NAD enhances glycolysis via glyceraldehyde 3-phosphate dehydrogenase and lactate dehydrogenase, which require NAD as a coenzyme, and thus continuous replenishment of NAD supports fast-growing cancer cells.[4] Besides its critical role as a coenzyme in metabolism, NAD is a substrate of poly(ADP-ribose) polymerase (PARP) and Sirtuins, mediating the NAD-dependent poly-ADP ribosylation and deacetylation, respectively. Because of both functions, NAD is involved in several key signaling pathways that are often altered in cancer, such as cell cycle progression, DNA repair, and metabolic regulation. Therefore, it is conceivable that limiting the availability of NAD would counteract pathways promoting cancer cell survival. The salvage pathway is considered critical in maintaining intracellular levels of NAD.[3] Nicotinamide phosphoribosyltransferase (NAMPT) is the major rate-limiting enzyme for NAD biosynthesis, and several specific NAMPT inhibitors have been investigated in both preclinical and clinical trial settings to develop potential anticancer therapeutics.[5–7]

In this issue of *Neuro-Oncology*, Sharma et al investigated the role of NAMPT inhibition in regulating glioma cell proliferation and survival by using KPT9274, a newly developed NAMPT inhibitor.[8] First, the authors demonstrated that glioma cell viability was significantly suppressed by both genetic knockout and pharmacological inhibition of NAMPT, suggesting that NAMPT expression is essential for glioma cell survival. In order to explore the impact of the NAMPT inhibition on a heterogenous tumor, they deliberately factored in several key biomarkers when selecting glioma cell models for the study, so that the cell lines represent gliomas with a variety of genetic backgrounds and distinct biological features, including *MGMT* promoter methylation and *IDH* mutation. The authors further demonstrated reduced cell proliferation rate, apoptosis induction, and angiogenesis inhibition in their cell models following KPT2974-induced NAMPT inhibition. In addition, they demonstrated reduced sphere formation and self-renewal ability of resistant glioma stem-like cells (GSCs). They then examined the effects of KPT2974 on glioma cell metabolism and found a significant reduction in basal respiration, respiration capacity, and active cellular mitochondrial content via Seahorse assay and MitoTracker staining, respectively, indicating that KPT2974 induces mitochondrial dysfunction. The KPT9274-induced depletion of NAD, adenosine monophosphate, and deoxyguanosine monophosphate, from the results of a global metabolomic analysis of a GSC line, confirmed the direct inhibitory effect of KPT9274 on the NAD salvage pathway. Furthermore, the decrease in NADH and NADPH and increase in ROS (reactive oxygen species) production and DNA damage were observed in KPT9274-treated glioma cells. Finally, the reduction in NAD in tumor tissues, resulting from inhibition of NAMPT, was confirmed by using viable organotypic mouse and human glioma tissues. Downregulation of Sirtuin1 and induction of cleaved PARP were demonstrated in the ex vivo glioma model and were consistent with findings from the in vitro models. Overall, Sharma et al's results demonstrate that KPT9274-induced NAMPT inhibition causes mitochondrial dysfunction and subsequent ROS overload, DNA damage, and apoptosis, independent of tumor genetic subtypes, indicating the therapeutic potential of NAMPT inhibitors in treating gliomas.

While the preclinical data of NAMPT inhibition in cancers have been promising, the implementation of NAMPT inhibitors in clinical trials remains challenging. Dose-limiting hematological toxicities, such as thrombocytopenia and other non-hematological toxicities, have been reported in early phase clinical trials investigating NAMPT inhibitors.[6,7] The systemic toxicities may stem from the need for NAD in normal organ tissues. Sharma et al gave meticulous attention to the therapeutic window of KPT9274.[8] NAMPT expression levels were found to be elevated in glioma cells when compared to normal human astrocytes (NHA), and the cell viability of NHA cells and human brain microvascular endothelial cells was less affected by the KPT9274. Furthermore, NAD and ATP levels remained unchanged in NHA cells following KPT9274 treatment, suggesting a potential therapeutic window of KPT9274 as a treatment for gliomas. Regardless, the sensitivity to NAD-depleting therapy may vary in other normal organ tissues, thus causing systemic toxicities. One way to increase sensitivity to the drug and minimize side effects is to identify and treat the tumors with high selective vulnerability to NAD-depleting treatment. For example, nicotinate phosphoribosyltransferase (NAPRT) is another enzyme involved in the synthesis of NAD. A lower level of NAPRT renders tumor cells solely dependent on NAMPT in order to replenish the NAD pool. It has been shown that the loss of NAPRT expression, often through promoter methylation, enhances the efficacy of NAMPT.[9] While Sharma et al demonstrated broad antitumor activity in both IDH-mutant and IDH-wildtype gliomas, another group showed that IDH-mutant gliomas have lower NAPRT expression, which resulted in an increased sensitivity to NAMPT inhibition.[10]

Finally, Sharma et al noted that despite strong in vitro and ex vivo anti-glioma effects, KPT2974 has limited blood-brain barrier (BBB) penetration, leading to ongoing efforts to improve drug delivery methods.[8] Taken together, pharmacological inhibition of NAMPT holds great promise in treating gliomas. Clinical investigation of NAMPT inhibitors with better BBB penetration along with strategies to minimize systemic toxicities will largely improve glioma treatments.

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