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AUTHOR'S VIEWS

Dual role of N-acetyl-aspartyl-glutamate metabolism in cancer monitor and therapy

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ABSTRACT

We uncovered the neurotransmitter N-acetyl-aspartyl-glutamate (NAAG) as a reservoir providing glutamate to promote cancer growth, and demonstrated that inhibition of NAAG hydrolysis by targeting glutamate carboxypeptidase II is a viable strategy for cancer therapy. Our study also suggests that NAAG concentration in plasma could be a non-invasive measurement to monitor cancer progression.

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Over the last few decades, creating effective therapies for advanced-stage cancers has been the main focus of many research studies. In this context, studying the metabolism of cancer cells has been explored as a new research avenue for novel treatment strategies. We, and others, have established that glutamine metabolism plays an important role in cancer proliferation.[1–3] This dependency of cancer on glutamine could be exploited to develop new therapies for cancer treatment. However, the current clinical trials to block the conversion of glutamine to glutamate via pharmacological inhibition of glutaminase have provided limited clinical efficacy.[4] Aiming to address these limitations by targeting other pathways, we employed mass-spectroscopy-based stable isotope-resolved metabolomics (SIRM) with $^{13}C_5{}^{15}N_2$-labeled-glutamine to explore glutamine metabolism beyond glutaminolysis. This investigation led us to discover an interesting phenomenon pertaining to the neurotransmitter metabolite, N-acetyl-aspartyl-glutamate (NAAG) in cancers. In particular, we observed a significantly higher production of NAAG from glutamine in higher-grade cancers compared to their lower-grade counterparts in three different models (*in vitro, in vivo* and patient samples) of three different cancer types (lymphoma, ovarian and brain tumors).[5] Moreover, in brain tumors, the NAAG concentrations in patient plasma strongly reflected those in tumors. Furthermore, we discovered that NAAG concentrations in plasma mirrored tumor growth in the mice bearing human lymphoma tumors. NAAG concentration spikes were detected prior to any surges in tumor growth. The significant elevation of NAAG in higher-grade cancers and the strong mirrored relationship between NAAG in plasmas and tumor growth have shown the clinically relevant potential of NAAG, paving the path toward developing non-invasive cancer monitoring measurement. Specifically, this finding can be used for the future prospective approach of measuring the NAAG levels in peripheral blood plasma for real-time monitoring of tumor growth (Figure 1). In an effort to translate this finding into a clinically relevant application, we plan to further expand into other cancer types and use NAAG concentration in plasma in combination with current indicators of cancer (stage of cancer, grade of cancer, age, other factors) to develop a more cohesive understanding of the progression of cancer in patients.

In parallel with the efforts to develop non-invasive measurement of NAAG concentrations for tumor progression assessment, we were dedicated to elucidating the mechanisms behind the upregulation of NAAG in promoting cancer growth as this understanding would contribute to tackling cancers from various angles to help improve therapeutic outcomes. NAAG is a well-investigated neurotransmitter in several neurological disorders.[6] However, its role in cancer is still unclear. Long et al. reported a possible role of NAAG as an inhibitory factor for differentiation of glioma stem-like cells.[7] Furthermore, a global metabolomics profiling of ovarian cancer (OVCA) showed that NAAG and NAA (N-acetyl-aspartate) levels were more elevated in metastatic OVCA than in primary OVCA or normal ovary, but did not provide the specific role of these metabolites.[8] With the use of $^{15}N_2$-labeled-NAAG, we demonstrated that the hydrolysis of NAAG directly produced $^{15}N_1$-glutamate *in vivo* via glutamate carboxypeptidase II (GCPII). Glutamate is not only essential for bioenergy synthesis and redox homeostasis but also nucleotide synthesis precursors for DNA synthesis.[9] For these reasons, the current clinical trial aims to reduce glutamate production by inhibition of glutaminase, the enzyme that converts glutamine to glutamate (Figure 1). Given our finding that NAAG can hydrolyze to glutamate in tumor expressing GCPII, we chose 2-PMPA (2-(Phosphonomethyl)-pentandioic acid, 2-Phosphonomethyl pentanedioic acid), a specific GCPII inhibitor with the greatest binding affinity (IC50 = 0.3nM)[6] to stop NAAG from hydrolyzing to glutamate. We found that inhibition of GCPII reduced tumor

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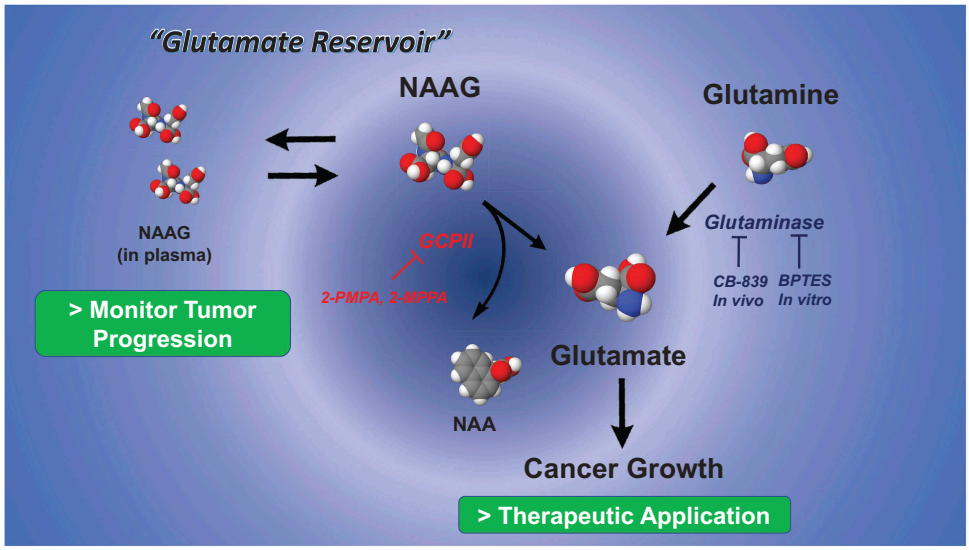

Figure 1. Dual role of N-acetyl-aspartyl-glutamate (NAAG) metabolism in cancer monitor and therapy. NAAG concentration in plasma can be a non-invasive measurement to monitor cancer growth. Blocking glutamate carboxypeptidase II (GCPII) in combination with glutaminase inhibition synergistically reduces production of glutamate. NAA: N-acetyl-aspartate, BPTES (bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulphide), CB-839 (2-(pyridin-2-yl)-N-(5-(4-(6-(2-(3-(trifluoromethoxy)phenyl)acetamido)pyridazin-3-yl)butyl)-1,3,4-thiadiazol-2-yl)acetamide): glutaminase inhibitor, 2-PMPA (2-(Phosphonomethyl)-pentandioic acid, 2-Phosphonomethyl pentanedioic acid), 2MPPA (2-(3-Mercaptopropyl)pentanedioic acid): GCPII inhibitor.

growth in patient-derived recurrent ovarian cancer orthotopic tumors in and suppressed glutamate production. Furthermore, we believe that simultaneously targeting these glutamate-supplying pathways in cancer would yield better outcomes. Thus, we combined 2-PMPA with CB-839 (2-(pyridin-2-yl)-N-(5-(4-(6-(2-(3-(trifluoromethoxy)phenyl)acetamido)pyridazin-3-yl)butyl)-1,3,4-thiadiazol-2-yl)acetamide), a current clinical trial glutaminase inhibitor,[4] and observed that tumor growth was significantly more suppressed under the combination treatment as compared to either treatment alone (Figure 1). The fundamental rationale for the significant reduction in tumor growth in the combined treatment was the synergistically-reduced amount of glutamate. The therapeutic approach of our study, including the new finding of NAAG metabolism in cancer, provides a significant improvement to the therapeutic index as they target glutamine metabolism from several angles. This encouraging result not only demonstrates the significance of NAAG in providing glutamate via a GCPII dependent pathway but also partly addresses the clinical limitation of the current clinical trial of CB-839. Of note, the oral form GCPII inhibitor, 2-MPPA (2-(3-Mercaptopropyl)pentanedioic acid), has passed a Phase I clinical trial for other diseases.[10] With the availability of these two clinical-trial inhibitors, the translation of targeting two important glutamate-producing pathways into cancer therapy appears to be promising.

In conclusion, NAAG is shown to be not only a potential metabolite marker for cancer motoring but also a glutamate provider to support cancer growth. This dual role of NAAG can be translated into clinical applications. Moreover, targeting NAAG metabolism could also be employed in combination with other pharmacological approaches such as CB-839 to improve clinical outcomes. The metabolomics-based discovery of metabolic aspects of cancer provides a unique tool to identify and guide specific targeted pharmacological approaches. Our innovative metabolomics-based insights allow for the uncovering of clinically-relevant biological processes and unveil new metabolic targets in cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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