Mutant isocitrate dehydrogenase 1/2 inhibition induces a unique MRS-detectable metabolic signature in low-grade gliomas

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Highlights:

- IDHmut inhibition induces a drop in 2-HG, and an increase in glutamate and phosphocholine in glioma cell models
- The flux from extracellular glutamine to intracellular glutamate increases following IDHmut inhibition
- This unique MRS-detectable metabolic profile can potentially be exploited for early non-invasive, clinically translatable detection of response to IDHmut inhibitors

Gliomas are the most common type of brain tumor in adults, representing 80% of all primary malignant central nervous system tumors. Mutations in the cytosolic enzyme isocitrate dehydrogenase 1/2 (IDHmut) are reported in 70-90% of low-grade gliomas and secondary glioblastomas. The wild-type isocitrate dehydrogenase (IDHwt) enzyme is important for cellular respiration and converts isocitrate to α-ketoglutarate (α-KG). Mutations most commonly occur at the R132 residue in the active site of IDHwt and lead to the neomorphic reduction of α-KG to 2-hydroxyglutarate (2-HG). 2-HG is an oncometabolite that ultimately drives tumorigenesis. Inhibition of IDHmut is therefore an attractive therapeutic approach and targeted inhibitors of IDH1 (AG-120) and pan-IDH1/2 (AG-881) have shown promising results in phase 1 and 2 clinical studies for gliomas. There is an urgent need to identify non-invasive methods of imaging response to AG-120 and AG-881. Prior work from our laboratory has also revealed the role of 2-HG in inducing magnetic resonance spectroscopy (MRS)-detectable metabolic reprogramming in IDHmut glioma cells. Therefore, the goal of this study was to examine the utility of MRS to non-invasively image response to IDHmut inhibition in low-grade gliomas. To this end, we used ^{1}H and ^{13}C-MRS to investigate the response of two genetically-engineered IDHmut cell lines (U87-based and normal human astrocyte (NHA)-based) to AG-120 and AG-881 treatment. As expected, in both cell lines, our ^{1}H-MRS data indicated that AG-120 and AG-881 induced a significant decrease in 2-HG. Interestingly, consistent with previous data linking 2-HG to reduced glutamate and phosphocholine levels, we observed a significant increase in phosphocholine and glutamate following treatment with AG-120 and AG-881. These results point to a unique MRS-detectable signature of IDHmut inhibition. To further investigate the mechanism behind the increase in glutamate levels induced by IDHmut inhibition in our models, we used ^{13}C-MRS to examine the flux from [1-^{13}C] glucose or [3-^{13}C] glutamine to ^{13}C-labeled glutamate. In the NHA model, we observed significant increase in the flux of [3-^{13}C] glutamine to ^{13}C-glutamate following IDHmut inhibition. In contrast, the flux of [1-^{13}C] glucose to ^{13}C-glutamate remained unchanged. In the U87 cell model, we also saw a significant increase in the flux of [3-^{13}C] glutamine to glutamate following treatment. However, we also saw a slight increase in the flux of [1-^{13}C] glucose to glutamate in this model. Since the increase in glutamine-derived glutamate is common to both models, it is likely to be a robust biomarker of response to therapy. Based on these results, we plan to explore the utility of monitoring the flux of hyperpolarized [1-^{13}C] glutamine or

hyperpolarized [1-^{13}C] α-KG to 2-HG as a means of measuring response to IDHmut inhibition. We also plan to examine whether the flux of hyperpolarized [1-^{13}C] glutamate to hyperpolarized [1-^{13}C] α-KG or the flux of hyperpolarized [2-^{13}C] pyruvate to hyperpolarized [5-^{13}C] glutamate can probe response to IDHmut therapy. Taken together, our studies indicate that IDHmut inhibition induces a unique MRS-detectable metabolic profile that can potentially be exploited for early non-invasive, clinically translatable detection of response to emerging IDHmut inhibitors.

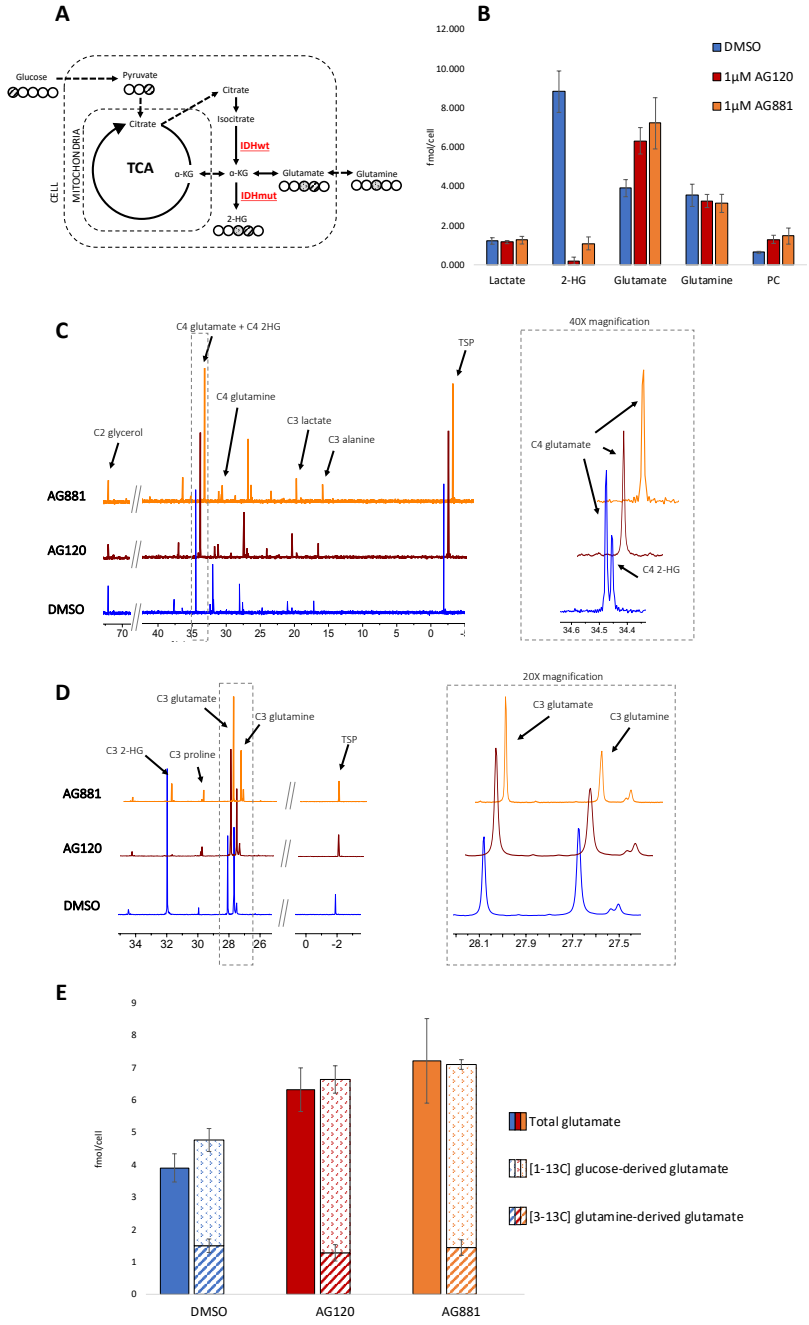

Figure 1. IDHmut inhibition induces a unique MRS-detectable metabolic profile. **(A)** Schematic pathway illustrating role of IDHwt and IDHmut, as well as ^{13}C-labeling of glutamate derived from [1-^{13}C] glucose and [3-^{13}C] glutamine. **(B)** Quantification of metabolite levels in NHAmut cell extracts following treatment, quantified using ^{1}H-NMR. **(C)** Representative ^{13}C-NMR spectra of [1-^{13}C] glucose-labeled cell extracts. **(D)** Representative ^{13}C-NMR spectra of [3-^{13}C] glutamine-labeled cell extracts. **(E)** Quantification of glutamate produced from [1-^{13}C] glucose and [3-^{13}C] glutamine and total glutamate levels in NHAmut cell extracts.