13C MRS shows that mutant IDH1 glioma cells alter flux through pyruvate dehydrogenase and pyruvate carboxylase

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Target Audience

NMR applications in neurooncology, metabolic reprogramming in cancer

Purpose

Gliomas are CNS malignancies that can be divided into high-grade and low-grade. A majority of low-grade gliomas carry an R132H mutation in IDH1, the cytosolic isocitrate dehydrogenase gene. The wild-type enzyme converts isocitrate to alpha-ketoglutarate (α-KG), whereas the mutant enzyme converts α-KG to R-2-hydroxyglutarate (2-HG), an oncometabolite that modulates several α-KG-dependent enzymes and leads to tumorigenesis.[1]

In normal astrocytes, pyruvate dehydrogenase (PDH) uses mitochondrial pyruvate to generate acetyl-CoA; it is strictly energetic, contributing no net carbons to the TCA cycle. In contrast, anaplerosis through pyruvate carboxylase (PC) is required to regenerate TCA cycle intermediates lost in the production of neurotransmitters, namely glutamate and GABA. Here, we explore the balance of metabolic flux through these two enzymes in low-grade gliomas harboring the IDH1 mutation.

Methods

Cell lines: U87 glioblastoma cells were transduced with either wild-type IDH1 or mutant IDH1 (IDHmut). Normal human astrocytes (NHAs), transduced with E6/E7/h-TERT to confer immortality[2], were similarly transduced with wild-type or mutant IDH1.

Flux analysis: U87 and NHA cells were grown in DMEM in which the glucose (normally 4.5g/L) was replaced with 1g/L of 2-^{13}C-glucose for 18hrs. Cell pellets were extracted using the dual-phase extraction. The aqueous phase was lyophilized, resuspended in D_2O, and analyzed using a 500MHz Bruker Avance spectrometer equipped with a TCI CryoProbe. Proton-decoupled ^{13}C spectra were obtained using a 30° flip angle and 3s relaxation delay averaged over 2048 acquisitions. Correction factors obtained from fully relaxed ^{1}H-coupled ^{13}C spectra were used to correct for saturation and NOE. The labeling pattern of glutamate was used to determine flux through PDH vs. PC using the equation[3]:

$$\frac{PDH}{PC} = \frac{2 \times [1]glu + [5]glu}{[2]glu + [3]glu}$$

where [1]glu is 1-^{13}C-labeled glutamate, [5]glu is 5-^{13}C-labeled glutamate etc.

Gene expression analysis: RNA was isolated using Rneasy kit (Qiagen) from cells. Q-RT-PCR was performed (UCSF Genome Core) using Taqman primers (Life Technologies). Expression was normalized to GAPDH transcript.

PDH activity and phosphorylation assays: PDH activity and phosphorylation assays were run according to manufacturer's instructions (Abcam). Activity was normalized per mg of protein. Phosphorylation was normalized to Janus Green stain for cell number.

PC activity: Cell lysates, containing cellular PC, were added to a reaction mixture containing $MgCl_2$, $NaHCO_3$, pyruvate, ATP, acetyl-CoA, and citrate synthase. Production of CoA was monitored using DTNB reduction, observed at 412nm, and normalized to time and protein.

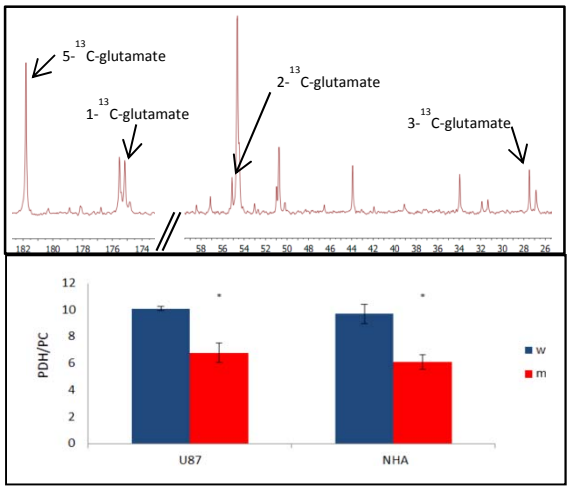

Figure 1 Top: Representative spectrum with glutamate peaks, used to calculate PDH/PC flux ratios. Bottom: PDH/PC flux ratios in mutant (red) and wild-type (blue) IDH1 U87 and NHA cell lines.

Results

In both U87 and NHA IDHmut cells, glutamate labeling patterns demonstrated a significant 32.5% ± 8.6% ($p<0.01$, n=3) and 37.1% ± 12.8% ($p<0.01$, n=3) decrease, respectively, in PDH/PC flux ratio. (Figure 1)

Correspondingly, PDH expression and activity levels decreased, while PC expression and activity levels increased significantly. In addition, inhibitory phosphorylation of PDH at Ser293 and Ser300 increased significantly in both IDHmut cell lines (Figure 2).

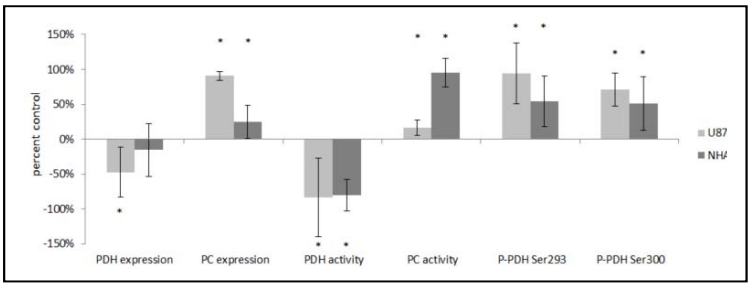

Figure 2: PDH expression, PC expression, PDH activity, PC activity and inhibitory phosphorylation of PDH, in IDHmut U87 (light grey) and NHA (dark grey) compared to wild-type.

Discussion

The decreased PDH/PC ratio indicates that a smaller proportion of pyruvate goes through PDH when compared to PC in IDHmut cells. Correspondingly, the activity of PDH decreased, consistent with both decreases in transcript expression and increases in inhibitory phosphorylation. Interestingly, the activity and expression of PC also increased. In U87 cells, the greater magnitude of change in PDH suggests it may be the bigger player. In NHA cells, the magnitude of both changes is comparable, suggesting regulation of both enzymes contributes to the decreased PDH/PC flux ratio. Taken together, this study reveals potential sites of metabolic reprogramming involving the fate of mitochondrial pyruvate in IDH1 mutant gliomas.

References

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Funding

NIH R21CA161545, NIH R01CA172845, NIH R01CA154915, NIH P41EB013598