**IDH1 mutated gliomas exhibit a distinct 31P MRS profile**

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**Purpose:** A large subset of glioma patients carries a mutation in the isocitrate dehydrogenase 1 (IDH1) gene1, which correlates with good prognosis. IDH1 mutated tumors produce the onco-metabolite 2-hydroxyglutarate (2HG) which can be detected by 1H MRS. As IDH1 regulates several pathways towards lipid synthesis2 we hypothesized that IDH1 mutant tumors will have an altered phospholipid profile. To test this, we performed 31P MRSI of human glioma xenografts growing in the mouse brain, one carrying the IDH1 mutation, 1H NMR of extracts of these tumors and of glioma cell lines expressing recombinant IDH1 with and without the mutation, and finally 31P HR MAS of biopsies of human gliomas with and without the mutation.

**Methods:** The following orthotopic glioma xenograft models were used: GBM-E473 (n=5) and GBM-E468 (n=5) (glioblastoma-derived), and Oligo-E434 (n=5) and Oligo-E478 (n=4) (oligodendroglioma-derived) of which the latter carries the R132H mutation in IDH1. U251MG glioma cells stably expressing IDH1 or IDH1-R132H were grown in glucose containing DMEM. Surgical biopsies were obtained from patients with (n=5) and without (n=6) IDH1 mutation.

In vivo 31P MR spectra were acquired on a 7T MR system (ClinScan, Bruker BioSpin) using a homebuilt quadrature coil and 3D pulse-acquire MRSI with an adiabatic 45˚ excitation pulse, TR 1500 ms, nominal voxel size 27 μl. MR spectra were analyzed by jMRUI. 31P NMR spectra of extracts were acquired on a Bruker Avance III, equipped with a multinuclear cryoprobe operating at 243.5 MHz. 31P HR MAS spectra were acquired on a Bruker Avance III 600 MHz, equipped with a 1H/13C/31P MAS probe.

**Results:** All IDH1 mutated tumors or cells showed 1H MRS signals for 2HG (not shown). Localized 31P MR spectra were acquired from each human glioma line (Fig. 1D) with good spatial resolution (Fig. 1A-C). The IDH1mutated E478 model was distinguishable from the IDH1wt tumors by significantly higher PC/PE and GPC/GPE ratios (Fig. 1E). This 31P-spectral profile of the IDH1-mutated model was verified by an analysis of extracted tumor tissues (Fig. 1E, "ex vivo"). 31P HR MAS spectra of human surgical biopsies identified that the same typical pattern occurs in IDH1 mutated tumors (Fig. 1F). Finally, we observed the same ratio changes in extracts of cell lines expressing mutated IDH1, subjected to in vitro 31P MRS (Fig. 1G).

**Discussion and Conclusion:** IDH1 mutations in gliomas are associated with a better prognosis and unique clinical behavior1. Here, for the first time, we demonstrate a typical 31P lipid spectral pattern apparently strongly associated with the IDH1 mutation in gliomas. The key observation is a depletion of P-ethanolamine compounds and apparent increase in P-Choline compounds. An increase in GPC levels has been reported for oligodendroglioma cells with the IDH1 mutation1. The metabolite ratios GPC/GPE and PC/PE can be used as alternative or complementary biomarkers in the diagnosis of IDH1 mutations as the detection of 2HG in 1H MRS is hampered by spectral overlap.

It is not clear why the IDH1 mutation causes a distinct change in the balance between the ethanolamine and choline branches of the Kennedy pathway. Because IDH1 plays various roles in cellular lipogenesis it is not surprising that a change occurs in the underlying metabolism. This may happen because of reduced αK and NADPH levels or because the tumor cells acquired different needs for ethanolamine and choline compounds.

**References:**


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**Figure 1:** (A-C) Orthogonal T2-weighted images of a representative Oligo-E434 mouse brains. (D) Representative in vivo 31P MR spectra of two orthotopic oligodendroglioma xenografts obtained from voxxels of interest. PC and PE exhibit inverse relationship in spectra of IDH1 mutant Oligo-E478 tumor line (green spectrum) compared to other xenografts. (E) Phosphorylated metabolite ratios obtained from ex vivo 31P MR data of xenograft tissues support the in vivo results. (F) 31P HR MAS MR spectra of surgical biopsies from GBM patients. PC/PE and GPC/GPE ratio levels in IDH1+ glioma patients are consistent with preclinical results. (G) The 31P MR spectral patterns of U251 cell lines carrying the R132H mutation resemble the in vivo and ex vivo data (the green spectrum).