IDH1 mutated gliomas exhibit a distinct 31P MRS profile

Morteza Esmaeili[1], Bob C. Hamans[2], Anneke C. Navis[3], Remco V. Horssen[4], Tone F. Bathen[1], Ingrid S. Gribbestad[1], William P. Leenders[3], and Arend Heerschap[1,2]

[1]Department of Circulation and Medical Imaging, Norwegian University of Science and Technology (NTNU), Trondheim, Norway, [2]Departments of Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [3]Departments of Pathology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [4]Departments of Cell Biology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands

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

Methods: The following orthotopic glioma xenograft models were used[2]: 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 ^{31}P 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. ^{31}P NMR spectra of extracts were acquired on a Bruker Avance III, equipped with a multinuclear cryoprobe operating at 243.5 MHz. ^{31}P HR MAS spectra were acquired on a Bruker Avance III 600 MHz, equipped with a ^{1}H/^{13}C/^{31}P MAS probe.

Results: All IDH1 mutated tumors or cells showed ^{1}H MRS signals for 2HG (not shown). Localized ^{31}P 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 ^{31}P-spectral profile of the IDH1-mutated model was verified by an analysis of extracted tumor tissues (Fig. 1E, "*ex vivo*"). ^{31}P HR MAS 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* ^{31}P MRS (Fig. 1G).

Discussion and Conclusion: IDH mutations in gliomas are associated with a better prognosis and unique clinical behavior[1]. Here, for the first time, we demonstrate a typical ^{31}P 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 mutation[3]. 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 ^{1}H 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 αKG and NADPH levels or because the tumor cells acquired different needs for ethanolamine and choline compounds.

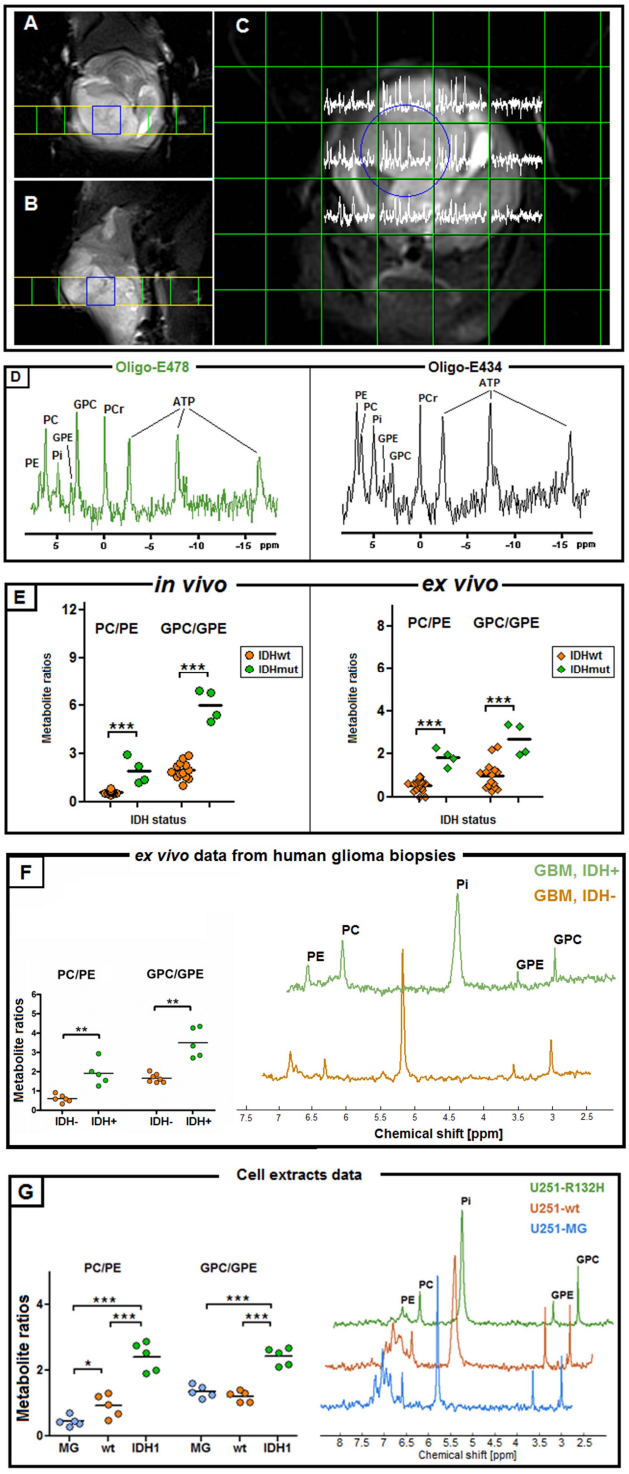

Figure 1: (A-C) Orthogonal T2-weighted images of a representative Oligo-E434 mouse brains. (D) Representative *in vivo* ^{31}P MR spectra of two orthotopic oligodendroglioma xenografts obtained from voxels of interest. PC and PE exhibit inverse relationship in spectra of IDH1 mutant Oligo-E478 tumor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

References: 1. Yang H, et al. IDH1 and IDH2 Mutations in Tumorigenesis: Mechanistic Insights and Clinical Perspectives. *Clin Cancer Res.* 2012;18(20):5562-5571. **2.** Claes A, et al. Phenotypic and genotypic characterization of orthotopic human glioma models and its relevance for the study of anti-glioma therapy. *Brain Pathol* 2008;18(3):423-433. **3.** Reitman ZJ, et al. Profiling the effects of isocitrate dehydrogenase 1 and 2 mutations on the cellular metabolome. *Proc Natl Acad Sci U S A.* 2011;108(8):3270-3275.