In vivo 3D ^{31}P MR spectroscopic imaging of human brain tumors growing orthotopic in the mouse

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Introduction: A widely accepted biomarker for brain tumors is the methyl signal of choline compounds in ^{1}H MR spectra [1]. Although the level of this peak correlates with tumor grade, it has little value in the discrimination of brain tumour types. A major reason may be that it is composed of different compounds: choline, phosphocholine (PC), glycerophosphocholine (GPC). In ^{31}P MRS the signals of P-metabolites can be observed separately and in addition enables to study other key compounds in the Kennedy pathway like phosphoethanolamine (PE) and glycerophosphoethanolamine (GPE), next to high energy P-compounds as ATP and PCr. Thus ^{31}P MRS *in vivo* is relevant to study tumor biology, to evaluate and improve treatments and to identify diagnostic biomarkers [2]. For these studies it would be very relevant also to be able to perform localized ^{31}P MRS of orthotopically growing brain tumors in small animals. Therefore, the aim of this study was to explore the feasibility to perform *in vivo* ^{31}P MR spectroscopic imaging of different human derived glioma growing in the mouse brain.

Methods: Different type of well-characterized orthotopic human glioma models were used [3]: Two glioblastomas (GBM-E473, GBM-E468) and two oligodendrogliomas (Oligo-E434, Oligo-E478). Mice were positioned in the prone position in a homebuilt stereotactic mouse holder and were anesthetized using 2 % isoflurane (Abott, Cham, Switzerland) in a 2:1 oxygen and N2O mixture. Body temperature and breathing were measured and controlled using an animal monitoring system (Small Animal Instruments Inc, NY, USA). MR experiments were performed on a 7T preclinical MR scanner interfaced to a clinical console (ClinScan, Bruker BioSpin, Ettlingen, Germany) using a homebuilt quadrature coil. The coil consisted of a quadrature polarized Tx/Rx ^{31}P coil, fitting tight around the mouse head to obtain an as high as possible SNR, encompassed by a bigger ^{1}H surface Tx/Rx coil for background imaging and shimming of the mouse brain.

^{31}P MR spectra were acquired using a 3D pulse-acquire CSI sequence, with an adiabatic 45˚ excitation pulse, TR=1500 ms, FOV=24x24x24mm and matrix size=8x8x8, resulting in a voxel size of 3x3x3mm and total acquisition time of 1:52 hour. Selected MR spectra within the mouse brain were analyzed using jMRUI software.

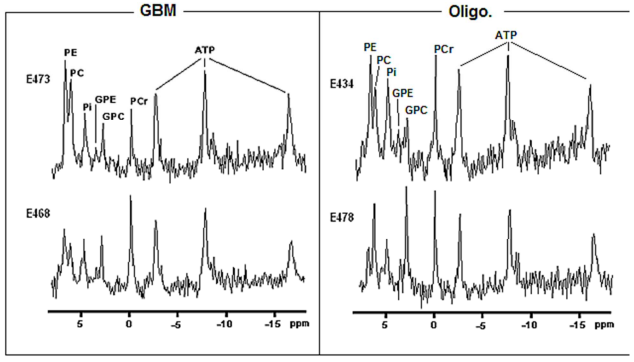

Fig. 2: ^{31}P MR spectra recorded from 4 orthotopic glioma xenograft tumor lines in mouse brain.

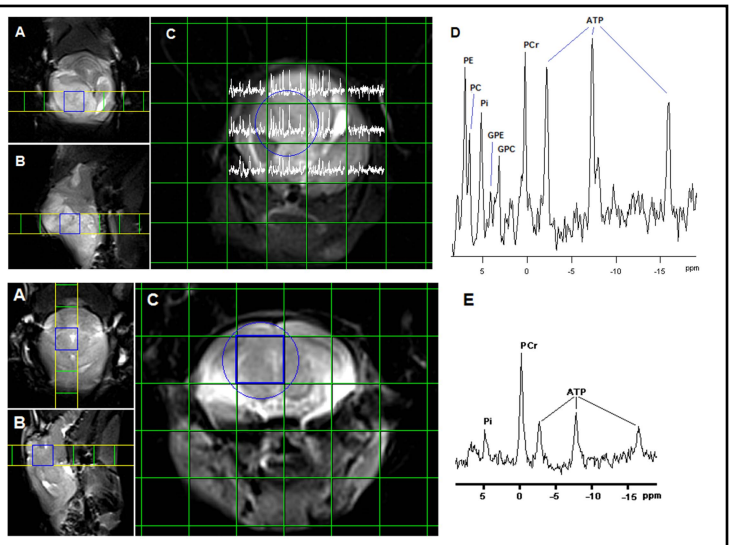

Fig. 1: (A-C) Orthogonal T2-weighted images of a brain with a Oligo-E434 (top) and healthy mice brains (bottom) in axial, sagital, and coronal view, respectively. (D-E) Corresponding ^{31}P MR spectra of a 27 µl voxel in tumor and healthy brain respectively.

Results: Excellent localized ^{31}P MR spectra could be acquired from each human brain tumour line, all distinctively different from those of the healthy brain (Figure 1). The Oligo-E478 glioma identified with a significant higher PC/PE and GPC/GPE ratios from the other lines (Figure 2). For all tumors the Ki-67 proliferation indices and survival times were determined, which revealed that GBM-E468 and Oligo-E478 tumor lines had a better prognosis. These models exhibit a significant lower PC/GPC and PE/GPE ratio than the other lines ($P < 0.001$) and higher Pi level.

Discussion and Conclusion: This study presents the first 3D ^{31}P MRSI of orthotopic human gliomas in mouse brain with high SNR and spectral resolution. We observed increased levels of PC and PE in brain tumors in agreement with human glioma studies [2]. In particular the more aggressive tumors are characterized by high levels of these compounds which is in agreement with increasing levels of choline kinase activity in higher grade tumors [4]. The Oligo-E478, which had a typical ^{31}P profile turned out to carry an IDH1 mutation. Thus ^{31}P MRSI can be used to study tumor biology and to assess new treatments in orthotopic mouse models of human gliomas.

References: 1. Glunde ea. 2011 Nat Rev Cancer 17: 835; 2. Hattingen ea 2011, Neuro-Oncol 13: 1349; 3. Claes A. ea, 2008, Brain Pathol., 423:33; 4. Righi ea 2009 NMR Biomed 22, 629