^{1}H-HRMAS of Small-Molecule-Metabolites in Adult Brain Tumours: Assignment, Quantification and Biomarker Determination.

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Introduction

In vivo ^{1}H MR spectra are dominated by large peaks (e.g. tCr, tCho, mI and lipids), which mask the contribution of smaller resonances (e.g. taurine, valine) that may provide diagnostic information on tumour type or treatment response. Although at low levels, signals from coupled spins may be amenable to detection *in vivo* by using appropriate editing sequences[1]. We have used 1D and 2D high-resolution magic angle spinning (HRMAS) ^{1}H NMR spectroscopy of brain tumour biopsy samples: 1) to provide an assessment of all the "NMR visible" small molecule metabolites that could potentially be detected *in vivo*; 2) to provide accurate peak assignments for tumour tissue metabolite quantification; 3) to determine metabolites that are potential biomarkers for particular tumour types.

Methods

Acquisition. Biopsy samples collected from 65 patients during surgical resection of their brain tumours and snap-frozen in liquid nitrogen were from: 5 grade II astrocytomas (4 diffuse and 1 gemistocytic astrocytoma), 5 grade III astrocytomas (3 anaplastic astrocytomas, 2 anaplastic oligoastrocytomas), 24 glioblastoma multiforme, 3 lymphomas, 8 metastases and 20 meningiomas. HRMAS was performed at 600MHz with 5 kHz spin rate at a sample temperature of 4°C. The HRMAS acquisition protocol included: presaturation pulse acquire with TR of 3.14 and 9.14s; CPMG with TE 50ms; 2D TOCSY with a 70 ms DIPSI-2 isotropic mixing sequence. ***Analysis.*** Average metabolite peak positions were determined from 1D and 2D spectra of the 5 tumour types. TARQUIN[2] was used to simulate a single basis set of spectra for LCModel analysis of all tumour types. Assignments of macromolecule signals from lipids and proteins were used for simulation of broad singlets with varying chemical shift and line width individually by LCModel. Metabolite concentrations were estimated using the unsuppressed tissue water signal as a reference.

Results

We determined that 29 (Ace, Ala, Asc, Asp, Bet, Cho, Cr, Glc, Glu, Gln, GSH, GPC, GPE, Gly, His, h-Tau, Ile, Lac, Leu, Lys, m-Ins, NAA, PCh, PE, s-Ins, Suc, Tau, Thr, Val) small molecule metabolites and 8 macromolecule signals accounted for the majority of the HRMAS spectrum of the eight types of brain tumours studied. The chemical shifts of individual peaks from 7 metabolites (Ala, Cr, Gly, Glu, Lac, PCho, Tau) were compared for 3 groups of tumours: meningioma, gliobalstoma and metastases. The peak linewidth was on average 7 times greater than the standard deviation of the peak position shift. There was no significant difference in peak positions (individual t-tests: $p > 0.05$) between tumour groups. Smaller LCModel residuals were obtained using simulated spectra with actual tumour tissue peak positions than using information from metabolite solution spectra.

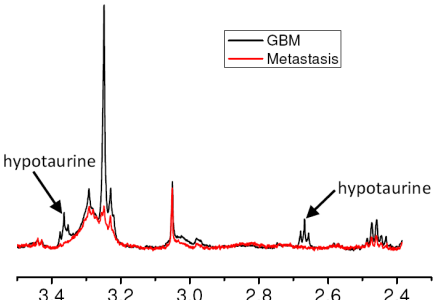

Fig. 1 Hypo-taurine significant for tumor type

Group 1	*Group 2*	*Significantly higher in group 1*	*Significantly higher in group 2*
Lymph	GBM	Tau*	
Lymph	Met.	h-Tau	
Lymph	Meng	Cr*	Ala**, Glu*, Gln**, Lac*
Ast II	GBM	m-Ins*	Ala
Ast II	Met	Gln*, m-Ins, s-Ins	PE
Ast II	Meng	Cr, GPC**, His**, m-Ins**, s-Ins**	Ala**, Glu*, GSH**, PE
Ast III	Met	h-Tau*	
Ast III	Meng	GPC*, m-Ins*	GSH*, Lys
GBM	Met	Cr, Gln*, Gly*, h-Tau*	
GBM	Meng	Asp, Cr**, GPC*, His*, m-Ins**, NAA*, s-Ins**	Ala, Glu*, GSH**, Ile*, Tau, Val*
Met	Meng	Cr*	Ala**, Glu*, Gln**, Lac*

Table 1 Binary group comparison of metabolite concentrations using Kruskall-Wallis test: ** $p < 0.001$; * $p < 0.005$; all other data $p < 0.01$.

There were significant metabolite differences between groups of different tumour types (Table 1). MANOVA analysis of metabolite concentrations was used to generate two discriminate functions that showed good separation of the tumour types: lymphoma, meningioma, metastases and gliomas.

Discussion

New assignments for brain tumour spectra were: ascorbate, betaine, GPE, histidine and hypotaurine (see Fig. 1). Significant levels of ascorbate were observed in all tumours except metastases. In particular, high concentrations of histidine and hypotaurine distinguish GBM from Met, and high concentrations of ascorbate, PE and valine distinguish lymphomas from other tumours. The increasing use of higher field MRI systems with specific editing may enable the future detection of these metabolites as tumour biomarkers.

References. 1. Terpstra M et al. 1H NMR detection of vitamin C in human brain in vivo**.** *Magn Reson Med* 2004, **51:**225-229. 2. Wilson M et al. High resolution magic angle spinning 1H NMR of childhood brain and nervous system tumours. *Mol Cancer* 2009, **8:**6.

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