In vivo and ex vivo 1H MRS metabolite profiles of gliomas

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Introduction

Knowledge of glioma grade is important for treatment selection and patient prognosis and ^{1}H MRS offers a uniquely non-invasive method of obtaining tumour biochemical profiles. Pattern recognition algorithms show most promise for robust tumour classification from ^{1}H spectra [1,2], whereas the metabolite ratios often used for determining tumour grade show significant overlap [3]. Astrocytoma growth and progression is heterogeneous and ratios do not require a reference, hence are useful for multi-voxel data analysis, and are independent of cell density and necrosis. However, necrosis is a marker for malignancy and cell density may also be relevant. We have used *in vivo* and *ex vivo* ^{1}H MRS with histopathology to quantify and model metabolite levels in gliomas to aid understanding of the variability of metabolite profile and its association with tumour grade.

Methods

Brain tumour patients had no previous treatment: 71 *in vivo* and 41 *ex vivo* ^{1}H spectra were obtained, with both data sets from 11 patients. Tumors were astrocytoma grades II (AS2), III (AS3) and IV (AS4) as determined from histopathology. *In vivo* ^{1}H MRS was performed on a GE 1.5T SIGNA with PROBE using PRESS or STEAM with TE 30 ms and TR 2000 ms. A single voxel acquisition was made from a tumour region avoiding cystic and necrotic regions as best possible. *Ex vivo* HRMAS ^{1}H NMR was performed on a 600 MHz Bruker Avance spectrometer and a water presaturation spectrum and tissue water signal acquired from each biopsy. Following HRMAS, the biopsy samples underwent histopathological analysis for cell density and percentage necrosis. LCModel analysis with a tissue water reference was used for quantification [1,4].

We investigated a four-compartment tumour model to describe the *in vivo* data consisting of fractions for viable tumour (F_v), hypoxic tumour (F_h), necrosis (F_n) and cyst (F_c). Each compartment was allowed to vary randomly in size with viable cells given a max metabolite concentration (Cho_{max}) and necrotic regions a maximum lipid concentration ($Lipid_{max}$) and scaled by a cell density (D): $tCho = Cho_{max}*(1 - F_n - F_c)*D$; $Lipids = (Lipid_{max}*[1 - F_v - F_c] + Lipid_{min})*D$; where $1 = F_v + F_h + F_n + F_c$.

Results

Histopathology indicated 14 AS2, 15 AS3 and 42 AS4 for *in vivo* analysis; 9 AS2, 3 AS3 and 29 AS4 for *ex vivo* analysis, of which 15 were non-necrotic; 6 low-grade and 5 high-grade tumours had both i*n vivo* and *ex vivo* spectra. Metabolite concentration variability was high; a typical standard deviation was 50% of the mean for low-grade and over 70% for high-grade tumours, with the exception of AS2 for which tCho variability was only 26% (Figs. 1 and 2). In AS4 there was a significant negative correlation between metabolite concentrations and 1.3 ppm lipids ($R \approx -0.5$, $p < 0.001$), with the data scattered but confined within a triangular region for *in vivo* (Fig. 3) and *ex vivo* data. Biopsy cellular density varied from 2500 to 8000 cells/mm^2 in non-necrotic biopsies, but metabolite levels did not correlate with cell density. There was good correlation between *in vivo* and *ex vivo* concentrations of mI, tCr, tCho and 1.3 ppm lipid ($R > 0.7$, $p < 0.005$, n=10 and one outlier removed). Fig. 4 shows a theoretical scatter plot of tCho versus lipids assuming a maximum 6 mM tCho and 400 mM lipid concentration as taken from Fig. 3, and a cellular density range taken from our *ex vivo* data. Inclusion of cystic regions assumed to contain no metabolites shifts the distribution towards the origin. Inclusion of hypoxic regions that maintain tCho levels but also have lipids present shifts the distribution away from the origin into the upper triangular section.

Figure 1. In vivo tCr and tCho variability

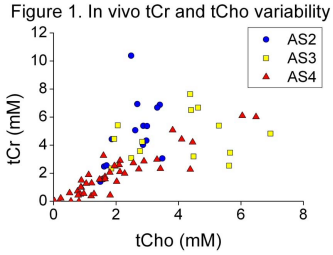


Figure 2. *Ex vivo* tCr and tCho variability

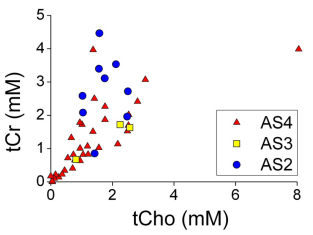


Figure 3. Metabolite variability in AS4

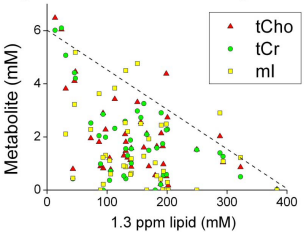
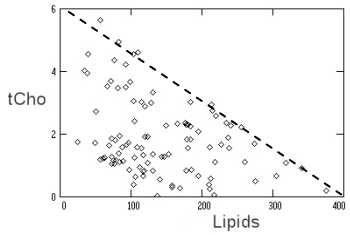


Figure 4. Simulated tCho variability in AS4



Discussion

Metabolite variability in AS2 and AS3 tumours does not appear to be dominated by cell density, and our data suggest that in AS2 there is greater variability in energy metabolism (high tCr variability) than in cellular proliferation (low tCho variability), whereas in AS3 there is high variability in both metabolism and proliferation. In AS4 the effect of necrosis is a significant factor that determines the measured metabolite concentrations, hence the high-correlation between tCho and tCr for AS4 in Fig. 1. We have previously shown that NMR visible lipid signals in human gliomas relate to lipid droplets [5] that are most likely formed during hypoxia prior to necrosis [6] and that these droplets then remain in the necrotic tissue. With lipid levels as a necrotic marker and tCho as a cellular marker, for $F_h = F_c = 0$ and constant cellular density, the simulated data in Fig. 4 would just show a diagonal of negative slope. Variability in cellular density creates a simulated distribution (Fig. 4) similar to that of real data (Fig. 3). The sparseness of data points in the upper triangle region of Fig. 3 is consistent with our simulation of a low fraction of cells in which lipid droplets have accumulated, and consistent with the idea that these cells rapidly progress to necrosis retaining the lipid droplets [5,6]. In conclusion, the 1.3 ppm lipid concentration is a robust biomarker for necrosis and hence for AS4 tumours. But the combination of metabolic and cellular variability is so great that single parameters such as a metabolite concentration or ratio cannot uniquely define AS3 or AS2 tumours, and pattern recognition methods utilizing all metabolite data should be used [1,2].

References

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