High-Resolution Magic-Angle Spinning ^{1}H Nuclear Magnetic Resonance Spectroscopy of Human Brain Tumors: Application of Pattern Recognition Method

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Introduction: Clinical data have shown that survival rates among brain tumor patients vary considerably depending on the type and the grade of the tumor. At present, the diagnosis of brain tumors relies mostly on histopathological evaluation on tumor biopsies. Recently, high-resolution magic-angle spinning proton (HR-MAS) ^{1}H magnetic resonance spectroscopy of brain tumor specimens has been shown to be potentially useful in tumor diagnosis and classification[1]. A large number of metabolites including N-acetyl aspartate (NAA), Choline (Cho), Creatine (Cre), Lactate (Lac), Alanine (Ala), Taurine (Tau), Glycine (Gly) and lipids (Lip) are observable by HR-MAS ^{1}H NMR, which not only can give rich information about tumor biology and metabolism, but also provide fingerprints for tumor classification. In this report, we present a preliminary study of 31 human brain tumors using HR-MAS ^{1}H NMR and principal components analysis (PCA).

Materials and Methods: Thirty one tumor specimens were obtained from 31 patients, among them there were 14 glioblastomase (3 medulloblastoma, 3 oligodendroglioma and 8 astrocytoma), 11 meningioma and 6 cases of haemangioblastoma and other hemorrhagic tumors. For the NMR experiments[2], the samples, weighting between 18 to 30 mg, were taken out from –85 ^{o}C refrigerator, rinsed in 0.9% D_2O saline on ice, and rapidly placed in a 4 mm rotor. HR-MAS ^{1}H NMR spectroscopy was carried out at 292 K using a 600 MHz Varian INOVA spectrometer. Spectra were acquired at a spin rate of 2 kHz and TR of 4 s. Typically, 128 transients were accumulated with 16k data points using the standard water pre-saturation pulse sequence and the standard Carr-Purcell-Meiboom-Gill (CPMG) spin-echo pulse sequence, with TE of 32 ms, a spectral width of 12000 Hz and an acquisition time of 0.667 s. HR-MAS spectra were phased and baseline-corrected using XWINNMR, and then the spectral region over the chemical shift range of 0.52-4.52 ppm in each spectrum was divided to 200 blocks, each having a width 0.02 ppm. The signal intensity in each block was integrated and normalized by dividing it by the sum of the integrals of all 200 blocks. PCA was carried out using mean-centered scaling with the software Simca-P 10.0. The results were visualized using PC scores plots, where each point on the scores plot represents an individual sample. Difference between the samples can be detected on the score plots. The spectral regions responsible for the difference can be viewed in the corresponding loading plots[3,4].

Results and Discussion: Figure 1 shows the HR-MAS ^{1}H NMR spectra from three typical tumors. There appeared to be specific spectroscopic patterns for different tumor types. For example, the resonance peaks of Cho and Cr were higher in medulloblastoma than in meningioma; the signal intensities of Ala and Lac were lower in medulloblastoma than in meningioma; NAA was hardly observable in all three tumor types; haemangioblastoma and hemorrhagic tumors were characterized by relatively broad lipids resonances at 1.27 ppm from $(-CH_2)_n$, at 0.87 ppm from $(-CH_3)$ and at 5.3 ppm from (-CH=CH-), which were not completely suppressed by the CPMG protocol and thus most likely came from the highly mobile lipids in the tumors. Figure 2 shows the PCA scores plots of all data from water pre-saturated spectra. It appeared on the plot that the brain tumors could be classified into three types, although, to some extent, overlap did exist among different tumor types.

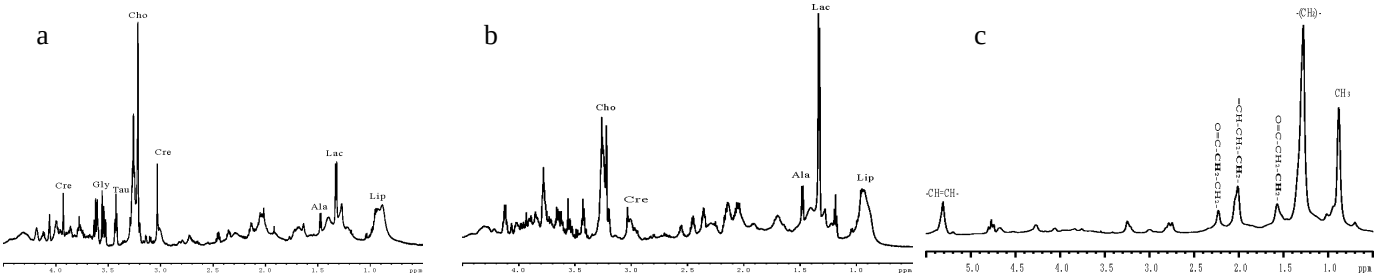

Figure 1. 600 MHz HR-MAS ^{1}H NMR spectra of different tumors at a rotation rate of 2 kHz. a) water pre-saturated spectrum of a medulloblastoma, b) water pre-saturated spectrum of a meningioma, c) CPMG spectrum of a haemangioblastoma.

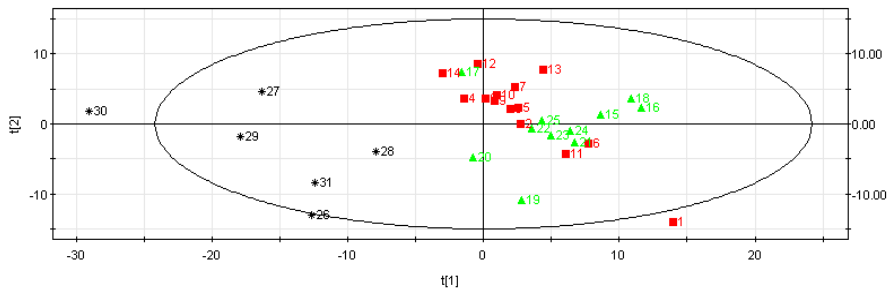

Figure 2. PCA scores plots (PC1 vs PC2) of HR-MAS ^{1}H NMR spectra of 31 human brain tumors. Red box: glioblastomase; Green triangle: meningioma; Black star: haemangioblastoma and other hemorrhagic tumors.

Conclusion: HR-MAS ^{1}H NMR spectroscopy combined with PCA is a useful technique for studying the metabolic changes in brain tumors. This technique not only can potentially be used to classify tumor types and tumor grades, and ultimately lead to the development of automatic diagnostic and cure methods, but also can provide valuable information for understanding the biology and pathology of the tumors.

Acknowledgements: Supported by the National Natural Science Foundation under the project number 10234070.

References: 1. L. L. Cheng et al, Cancer Res, 1998; 58:18525-1832; 2. N. J. Waters et al, Anal Biochem, 2000; 282:16-23; 3. Y. L. Wang et al, Anal Biochem, 2003 (in press); 4. J. C. Lindon et al, Prog Nucl Magn Reson Spec, 2001; 39:1-40.