Quantification of microheterogeneity in glioblastoma multiforme with ex vivo high-resolution magic-angle spinning (HRMAS) proton magnetic resonance spectroscopy¹

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Microheterogeneity is a routinely observed neuropathologic characteristic in brain tumor pathology. Although microheterogeneity is readily documented by routine histologic techniques, these techniques only measure tumor status at the time of biopsy or surgery and do not indicate likely tumor progression. A biochemical screening technique calibrated against pathologic standards would greatly assist in predicting tumor progression from its biological activity. Here we demonstrate for the first time that proton magnetic resonance spectroscopy (¹H MRS) with high-resolution magic-angle spinning (HRMAS), a technique introduced in 1997, can preserve tissue histopathologic features while producing well-resolved spectra of cellular metabolites in the identical intact tissue specimens. Observed biochemical alterations and tumor histopathologic characteristics can thus be correlated for the same surgical specimen, obviating the problems

caused by tumor microheterogeneity. We analyzed multiple specimens of a single human glioblastoma multiforme surgically removed from a 44-year-old patient. Each specimen was first measured with HRMAS ¹H MRS to determine tumor metabolites, then evaluated by quantitative histopathology. The concentrations of lactate and mobile lipids measured with HRMAS linearly reflected the percentage of tumor necrosis. Moreover, metabolic ratios of phosphorylcholine to choline correlated linearly with the percentage of the highly cellular malignant glioma. The quantification of tumor metabolic changes with HRMAS ¹H MRS, in conjunction with subsequent histopathology of the same tumor specimen, has the potential to further our knowledge of the biochemistry of tumor heterogeneity during development, and thus ultimately to improve our accuracy in diagnosing, characterizing, and evaluating tumor progression. *Neuro-Oncology* 2, 87-95, 2000 (Posted to *Neuro-Oncology* [serial online], Doc. 99-44, March 22, 2000. URL <neuro-oncology.mc.duke.edu>)

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³ Abbreviations used are as follows: GBM, glioblastoma multiforme;

¹H, proton; HRMAS, high-resolution MAS; MAS, magic-angle spinning; MR, magnetic resonance; MRS, MR spectroscopy; NMR, nuclear MR.

GBM,³ the highest grade of astrocytoma, is the most common malignant brain tumor currently found among adults (Levin et al., 1997). The extensive heterogeneity of these tumors, signified by the term "multiforme," has posed serious obstacles to the grading of tumor biopsies, especially in the case of small samples, and has hampered correlation of tumor biochemical changes with pathologic features. A nondestructive means of obtaining biochemical measurements in small tumor samples would allow a point-to-point correlation between biochemistry and tumor pathology.

Such a technique would also be of use in furthering our knowledge of the complexity of microheterogeneity found in GBM.

MRS possesses the sensitivity required to measure subtle biochemical changes. This technique may also have the potential to reveal the status of tumor microheterogeneity and to detect tumor metabolic mutations before they are clinically observable (Mountford et al., 1993). ^1H MRS studies of human brain tumors have been conducted both on excised intact tissues (Kuesel et al., 1994a, 1994b, 1996; Rutter et al., 1995; Somorjai et al., 1996) and, more commonly, on solutions of tissue extracts (Carpinelli et al., 1996; Florian et al., 1995; Kinoshita et al., 1994; Peeling and Sutherland, 1992; Sutton et al., 1994; Tugnoli et al., 1996; Usenius et al., 1994a, 1994b). These studies, which made use of conventional MRS techniques designed for the analysis of aqueous homogeneous solutions, sought to correlate neoplastic characteristics with ^1H MRS-measurable alterations in cellular metabolism. However, the fundamental physical differences between tissues and aqueous solutions have heretofore hampered the use of solution methodologies in studies of nonsolution tissues. These measurements produce broad-line, low-resolution spectra of limited use for either evaluating disease or quantifying tumor-related metabolic alterations. Moreover, the destructive nature of extraction alters measurable metabolites to an unknown degree, making correlations between extract MRS results and disease states questionable, even when the lengthy extraction procedures can be tolerated in clinical laboratories (Cheng et al., 1998a; Mountford et al., 1993).

To overcome the difficulties of conventional ^1H MRS in the analysis of tissue specimens, we developed a spectroscopic method that measures intact tissue with high spectral resolution (Cheng et al., 1996, 1997). This method uses MAS, a technique originally developed for the study of solid-state NMR, to rotate a specimen mechanically at a rate in the scale of kilohertz. This rotation creates an effect that mimics the rapid molecular motion seen in liquids, averaging the inhomogeneous characteristics in tissues and thus resulting in the reduction of spectral line width and improvement in resolution (Cheng et al., 1998a, 1998b; Garrod et al., 1999; Middleton et al., 1998; Millis et al., 1997; Moka et al., 1997, 1998; Tomlins et al., 1998; Weybright et al., 1998). In earlier studies, we demonstrated that highly resolved proton MR spectra of intact tissue specimens can be obtained by using high-resolution MAS ^1H MRS. In these studies, we correlated tissue metabolites, measured with HRMAS ^1H MRS, with disease states determined by histopathologic evaluation. These results, however, were obtained by studies of adjacent rather than identical tumor specimens (Cheng et al., 1996, 1998a, 1998b), whereas our earlier investigations were aimed at identifying differences between tumor classes. At that time, we made no attempt to address the issue of heterogeneity within a single tumor.

We have designed this study to test the capability of HRMAS ^1H MRS in the evaluation of tumor heterogeneity. In order to address the problem posed by tumor heterogeneity directly, while keeping the influence of

intersubject biological variability to its minimum, we evaluated 4 specimens, surgically selected from differing regions in a single tumor. HRMAS ^1H MRS was performed first, followed by quantitative histopathology. From the spectroscopy studies, we were able to define lactate levels between 20 and 80 mM and mobile lipid levels between 1 and 50 mM within the GBM. Subsequent histopathologic study of each tumor sample demonstrated that histopathologic features could be quantified after HRMAS ^1H MRS examination. Statistically significant linear correlations were found to exist between observed metabolic changes and the observed predominant histopathologic features. Our results suggest that HRMAS ^1H MRS may contribute greatly to the evaluation of tumor biochemistry and its correlation with histopathology and may augment our understanding of neuro-oncology at the level of metabolite activity. More importantly, because these spectral measurements were directly conducted with intact tissue, results thus obtained are closely related to the *in vivo* states of tumors. We therefore expect that the correlations observed between *ex vivo* MR spectra and histopathology will serve as powerful guidance for the future development of high resolution *in vivo* MRS and so permit the analysis of an entire tumor. The ability to examine the entire tumor, rather than a single small biopsy, should ultimately improve diagnostic accuracy and allow serial samplings.

Materials and Methods

This study involves the use of intact human brain tumor specimens. It was reviewed and approved by the Institutional Review Board at both the Massachusetts General Hospital and the Brigham and Women's Hospital, Boston, Massachusetts.

Collection of Tissue Specimens

A 44-year-old patient who developed headaches, vomiting, and deterioration of motor coordination with an expressive aphasia was scanned by MRI and found to have a left parietal mass. Biopsy of the mass indicated that the tumor was a GBM (World Health Organization grade IV) composed of cells with pleomorphic nuclei as well as areas of prominent pseudopalisading necrosis, mitoses, and vascular proliferation. The patient underwent surgical resection of the tumor. During the resection, a total of 4 tissue specimens were collected from 4 distinct tumor regions. In each region, 2 adjacent samples were obtained, 1 for routine histopathology, and 1 for use in this study, as outlined below.

Tissue specimens allocated for this study were snap-frozen in liquid nitrogen and maintained at -80°C until the time of spectroscopic analysis. Approximately one-half (between 53.5 and 60.0 mg) of each frozen specimen was weighed, positioned within a HRMAS rotor, and transferred into the NMR probe head, which had been precooled at 5°C . The HRMAS study was performed at this temperature and was started immediately at tissue thawing. After the MRS study, tumor specimens were

again frozen in liquid N₂, then submitted together with the unused fraction of frozen specimens for quantitative histopathologic examination.

¹H MRS

The spectroscopic procedures used for the HRMAS study have been described elsewhere (Cheng et al., 1997, 1998a). In brief, HRMAS experiments were performed at 5°C on an MSL400 NMR spectrometer (proton frequency at 400.13 MHz) by using a MAS probe (Bruker Instruments, Inc., Billerica, Mass.). Temperature was controlled by a VT-1000 unit in combination with a MAS pneumatic unit (Bruker Instruments). Sample spinning rate was stabilized at either 2.25 or 2.30 kHz for individual samples. A rotor-synchronized Carr-Purcell-Meibom-Gill pulse sequence [90-(τ -180- τ)_n-acquisition] was used to function as a T2-filter. The interpulse delay ($\tau = 2\pi/\omega_r$) was synchronized with the rotor rotation (τ indicates the sample spinning speed in time units and $\omega_r/2\pi$ represents the MAS speed in kilohertz). The value of n for each sample was adjusted according to sample spinning rates to create a T2 filter of $2n\tau = 20$ ms and 500 ms. The 90° pulse length varied from 10.5 to 12.1 μ s and was also adjusted individually for each sample. The number of transients was 256 for 20 ms and 512 for 500 ms T2 filters, respectively, with an acquisition time of 10 ms. A repetition time of 3.0 s and a spectral width of 8 kHz (20 ppm) was employed. Spin-spin relaxation time (T2) was measured by using the same Carr-Purcell-Meibom-Gill sequence with 64 transients and by varying n from 200 to 900.

All free induction decays were subjected to 1 Hz apodization before Fourier transformation and phase adjustment. Trimethylsilane at 0.00 ppm was used as an external chemical shift reference from which the internal reference of the lactate doublet was determined to be 1.32 and 1.34 ppm (Cheng et al., 1997, 1998a; Mountford et al., 1993; Sze and Jardetzky, 1990).

Tissue water was used as an internal standard to estimate the concentration of brain metabolites (Cheng et al., 1997, 1998a). The resonance of tissue water was measured without presaturation. The concentrations of metabolites in tissue specimens were estimated by using a formula previously given (Cheng et al., 1997). This estimation is based on the resonance intensities of metabolites and of tissue water from T2-filtered (both 20 ms and 500 ms) spectra, the T2 values measured for metabolites and water of each specimen, and an approximation that brain tissue contains 80% (weight) of water (44 mmol/g) (Cheng et al., 1997, 1998a; Usenius et al., 1994a).

Quantitative Histopathology

The paired frozen tissue samples (those subjected and not subjected to HRMAS analysis) of each specimen from 4 tumor regions were thawed, fixed in 10% formalin, embedded in paraffin, cut into 8- μ m sections, and stained with hematoxylin and eosin. Each specimen was entirely embedded, and a standard histopathologic slide of each sample was prepared. The cross-sectional area was approximately 30 mm² for HRMAS-measured samples. In

general, the tissue from the same site that was not measured by HRMAS ¹H MRS was significantly larger. The entire area of the tissue section was determined, and the percentage of the area composed of 6 types of tissue was measured by a neuropathologist (Dr. D.C. Anthony) to the nearest 5%. Six basic pathologic features were measured for each sample: areas of highly cellular glioma, with high cell density, mitoses, and other pathologic characteristics that indicate a GBM; areas of infiltrating glioma that were moderately cellular and clearly diagnostic of glioma, but did not have the high cellularity of the most malignant portions of the tumor; areas of nearly-normal brain tissue that contained normal resident cells and some reactive astrocytes but in which the number of infiltrating neoplastic cells was too low to establish a diagnosis of glioma; areas of necrosis, ranging from small foci of serpiginous zones of necrosis with pseudopalisading of tumor cells at periphery, to large zones of confluent necrosis; vascular structures, including bundles of collapsed meningeal vessels, large arteries, and zones of vascular proliferation; and areas of blood clot. The proportions of each of the 6 pathologic features were independently calculated as a percentage of the total area of specimen without knowledge of spectroscopic measurements on the specimen.

Results

Fig. 1 presents T2-filtered (500 ms) HRMAS ¹H MR spectra of the 4 GBM specimens excised from a single tumor and labeled A to D to denote the tumor region from which each specimen was obtained. Regional differences in cellular metabolism are clearly visible from these spectra.

Fig. 2 shows representative histopathologic images of the tissue specimens observed in regions A and D. The images on the left were obtained from specimens that were not subjected to HRMAS analyses, while those on the right were taken from corresponding samples located immediately adjacent and handled similarly, as described,

Fig. 1. Intact tissue high-resolution magic-angle spinning (HRMAS) proton magnetic resonance (¹H MR) spectra obtained from 4 different regions (labeled A–D) of a resected, untreated parietal glioblastoma multiforme (GBM) under a T2 filter of 500 ms. Alterations in tumor cellular metabolism for different regions are shown in these spectra, which reflect the metabolic heterogeneity of the GBM tumor. Lac, lactate; Pch, phosphorylcholine; Chol, choline; Cr, creatine; NAA, N-acetyl-aspartate; Acet, acetate.

Fig. 2. Examples of histopathologic images of GBM surgical specimens obtained from tumor regions A and D (labeled similarly to those in Fig. 1). Images on the left [AI (25), AI (100), and DI (100)], where the number in parentheses indicates the degree of magnification and the Roman numeral denotes tissue status] were obtained from standard neuropathologic analysis of specimens immediately adjacent to those subjected to HRMAS studies. Images on the right [AII (25), AII (100), and DII (100)] were obtained from specimens handled similarly as described but subjected to the HRMAS studies after thawing. At low-power magnification, both AI (25) and AII (25) have areas of nearly-normal cell density and foci of gliomas. In contrast, both DI (100) and DII (100) are largely necrotic. The histopathologic differences, such as apparent changes in nuclear shape, are frequently observed with tissues subjected to freeze/thaw. See Table 1 for details.

but evaluated first by HRMAS. The differences that are visible include apparent change in nuclear shape in AII (100), frequently observed in tissues that have been subjected to freeze/thaw, and the disappearance of hollow

structures in DII (100) in the necrotic tissue, likely compressed by the HRMAS g-force. These differences do not interfere with histopathologic determination of overall pathologic composition of tissue specimens.

Table 1. Quantitative neuropathologic analyses of four brain biopsy specimens obtained from a parietal GBM

Specimen	Section type ^a	Percent of area of entire cross-section				
		Glioma (highly cellular)	Glioma (moderately cellular)	Necrosis	Gliosis	Vascular tissue Blood clot
A	I		20		80	
	II		30	10	60	
B	I	10		30		60
	II	40		40		20
C	I	30		70		
	II		40	60		
D	I			100		
	II	10		90		

^aI, measured from standard neuropathologic analysis: routinely fixed in formalin, embedded in paraffin, cut in 8- μ m sections, and stained with hematoxylin and eosin. II, measured from specimen located immediately adjacent to I and handled similarly. However, these specimens were examined after thawing the frozen specimen that had been subjected to the high-resolution magic-angle spinning studies.

Table 1 summarizes the percentages of histopathologic features for each individual specimen from each region of the tumor, with and without HRMAS analysis. Data in this table show that adjacent specimens from the same tumor region share qualitatively similar histopathologic features, although quantitative differences were noted between them.

We observed a great extent of microheterogeneity in this tumor. Although all specimens provided for this study were classified as "tumor" and deemed appropriate for biochemical measurements by the attending surgeon, they were highly variable in their composition. Some samples were 90% to 100% necrotic, and thus might not provide much useful information about tumor cellular metabolism. Half of the samples contained no malignant tumor (AI, DI, in Table 1), and some contained only areas of adjacent infiltrating tumor of lower histologic grade (AI). Sample BI, although considered to be "tumor," was composed primarily of blood clot and necrotic tissue, with only 10% of the sample representing viable GBM.

We further quantitatively analyzed correlations between tumor metabolic concentrations in different regions and their corresponding histopathologic composition. For this purpose, we noticed that the area percentage of a particular histopathologic feature quantified from a randomly sectioned slide is equal to its volume percentage (Hyman et al., 1998). Of these correlations, we took particular note of the linear relationship between the concentration of mobile lipids and the amount of tumor necrosis. This relationship is seen in Fig. 3, which presents results from spectra of different T2 filter time (20 and 500 ms) for each specimen (see the Fig. legend for details). A similar, statistically significant correlation between measured concentration of lactate (a product of anaerobic respiration) and the amount of necrosis among different specimens is also seen in Fig. 4. In addition, in Fig. 5, we also observed a strong correlation between the amount of cancerous tissue (including both moderately and highly cellular areas, each of which contains pleomorphic and mitotically active cells) and the relative intensities of phosphorylcholine (3.22 ppm) to choline (3.20 ppm) from 500 ms T2-filtered spectra.

Discussion

Routine histopathologic evaluation of brain tumors, upon which diagnosis of GBM is based, relies on the evaluation of the following pathologic changes: atypia of nuclei, endothelial proliferation, cellular density, mitotic figures, and necrosis (Daumas-Duport et al., 1988; Levin et al., 1997; Yates, 1992). As the pathologic classification of brain tumors is inherently limited during life to small brain biopsies or partial resections, a number of alternative biologic and biochemical strategies for acquiring more extensive information about these tumors have been proposed (Coons and Johnson, 1993; Dalrymple et al., 1995; Hsu et al., 1997; Karkavelas et al., 1995; Landry et al., 1997; Leenstra et al., 1993; Montine et al., 1994; Sung et al., 1994; Weber et al., 1996). These methods aim to provide quantitative or semiquantitative evaluation of an entire human astrocytoma to improve the accuracy of patient prognostication. However, the extreme heterogeneity of GBM inherently confounds biologic, biochemical, or molecular procedures in cases where histopathology cannot be performed on precisely the same specimen.

The recent development of HRMAS ¹H MR spectroscopy creates a unique opportunity for evaluating tumor metabolism from a large specimen volume within a time frame of a few minutes. We have previously shown that metabolic changes measured with HRMAS ¹H MRS can be correlated with tumor types and grades as determined by routine histopathology for both human brain and breast cancer (Cheng et al., 1998a, 1998b). In this report, we present two additional findings that significantly advance our previous conclusions.

First, this study demonstrates that the procedure of HRMAS does not destroy the histopathologic characteristics of tissue. This fact allows us to quantify GBM metabolites and histopathologic features from the same specimen. The complication of tumor heterogeneity imposed on correlations established with two independent modalities is thus minimized. We believe that this protocol, which measures both tumor metabolism and histopathology from a single specimen, represents the first approach of its kind that can evaluate two sets of

Fig. 3. Correlations between the lipid intensities (0.90 ppm) and the amount of necrosis measured from the corresponding GBM specimens. Open circles (○) indicate the average concentration calculated for each tumor region from both spectra of 20 and 500 ms T2 filter time. A statistically significant linear correlation observed with least squares linear regression among data points (●) from spectra of 500 ms T2 filtering time is plotted. The vertical error bars represent the deviations of the value of each measurement (20 or 500 ms T2) from that of the average, and the horizontal error bars indicate the uncertainty in the histologic quantification of the amount of necrosis.

entirely independent parameters obtained with different methodologies from the same tumor specimen. Secondly, our results show that the well-known heterogeneous characteristics of GBMs can readily be quantified by HRMAS ^1H MRS. Our results also indicate that many metabolic alterations can be inferred from the corresponding histopathologic features. For instance, the known neuronal marker *N*-acetyl-aspartate (at 2.01 ppm) and its degradation product acetate (1.92 ppm) (Cheng et al., 1997) were not observed in three regions: B, C, and D. However, small amounts of *N*-acetyl-aspartate and acetate were detected in region A, which was largely composed of brain tissue with reactive changes rather than tumor (*N*-acetyl-aspartate concentration, 2.9 mmol/g versus ~12–14 mmol/g in normal human brain) (Barker et al., 1993; Cheng et al., 1997; Wang and Li, 1998). These neuron-specific resonances were observed in samples that consisted mostly of normal tissue; the presence of gliosis was the only histopathologic feature. It is thus very likely that the *N*-acetyl-aspartate signals observed here are related to the large number of axons in the neuropil.

The correlation between mobile lipids and the amount of necrosis, as shown in Fig. 3, is of importance due to the controversial role that tumor necrosis has played in the diagnosis of GBMs and its proposed prognostic value (Barker et al., 1996). This correlation suggests that the amount of the necrotic tissue in a specimen can be estimated from the measured concentration of lipids, ranging from 1 to 50 mmol/g. The data also indicate that these lipids are relatively mobile, for their T2s are about twice as long as those of tissue water (data not shown)

Fig. 4. A linear correlation between the lactate intensities (1.32 and 1.34 ppm) and the amount of necrosis measured from the corresponding GBM specimens. Data shown in the figure are the average concentration for each tumor region obtained from both spectra of 20- and 500-ms T2 filter time. This linear correlation is obtained with least squares linear regression analysis on the data points of the average concentration for each region. See Fig. 3 for definition of error bars.

and are likely to be the products of degenerated tumor cell membranes. This quantification, which extends our previous observation and supports the suggested relationship between mobile lipids and brain tumor necrosis (Gotsis et al., 1996; Kuesel et al., 1994b, 1996), can now be documented as a heterogeneous feature within a single tumor. We wish to emphasize that, in light of the importance of necrosis for the diagnosis of GBM, the HRMAS measurement of lipid resonances at high spectral resolution provides a unique opportunity to quantify these metabolites. Such quantification of lipids is extremely difficult to achieve with procedures of chemical extraction followed by conventional ^1H MRS.

In spite of confounding factors in relating the measured lactate concentrations with disease stages (Cheng et al., 1998a, 1998b), Fig. 4, showing a linear relationship observed between the measured lactate levels and the amount of necrosis in each region, agrees well with our previous conclusions on human malignancy studies and provides a context for them by indicating that the observed variation in lactate concentration may linearly reflect the amount of necrotic tissue in the analyzed specimens.

The strong correlation shown in Fig. 5 between the amount of cancerous tissue and the relative intensities of phosphorylcholine (3.22 ppm) to choline (3.20 ppm) was only observable with data points measured from 500 ms T2-filtered spectra. This metabolic ratio is difficult to measure on the 20-ms T2-filtered spectra, probably because of the inadequate resolution between these two important metabolites, which are significantly involved in cell proliferation at different stages of cancer development (Mountford et al., 1993; Ruiz-Cabello and Cohen, 1992). Almost all in vivo MR studies of adult brain tumors report a prominently increased signal in the

Fig. 5. A linear correlation between the proportion of gliomas (including both the highly and moderately cellular categories) and resonance ratios of phosphorylcholine to choline (Pch:Chol) measured from 500-ms T2-filtered spectra. This linear correlation was obtained with least squares linear regression analysis.

region that surrounds the 3.2-ppm area and corresponds to choline and phosphorylcholine resonances (Harada et al., 1995; Kugel et al., 1992; McBride et al., 1995; Miller et al., 1996; Preul et al., 1996; Shimizu et al., 1996; Sijens et al., 1994; Speck et al., 1996; Sutton et al., 1992; Useinius et al., 1994a, 1994b; Wang et al., 1995), although other metabolites may be presented as well. This signal is referred to in the literature as indicating "choline-containing metabolites;" however, neither the *in vivo* spectra nor the *ex vivo* spectra obtained by conventional NMR methods were able to distinguish which resonances contribute to the elevated signal (Kuesel et al., 1994a, 1994b; Rutter et al., 1995; Somorjai et al., 1996). Our observation suggests that the ratio of phosphorylcholine to choline (in Fig. 5) linearly correlates with the proportion of the specimens that is highly cellular. This correlation qualitatively agrees with results that we obtained in a human breast ductal carcinoma study and suggests that choline and phosphorylcholine resonances should be measured and quantified separately to yield more complete metabolic information pertinent to brain tumor classification. Accomplishing this goal *in vivo* will likely require both a better designed MRS pulse sequence for resonance editing and a higher magnetic field strength.

Finally, we tested the degree of correlations between tumor metabolites measured from one sample and the percentage of pathologic features evaluated from its adjacent sample from the same tumor region, as listed in

Table 1. Although we observed similar trends in relationships if lipid concentrations were plotted as a function of necrosis, a correlation coefficient of $r = 0.945$ was observed (similarly, lactate plotted against necrosis, $r = 0.949$; and phosphorylcholine/choline plotted against gliomas, $r = 0.747$), and the resultant correlations became statistically nonsignificant ($P = 0.055$, 0.051 , and 0.253 for lipid, lactate, and phosphorylcholine/choline, respectively). This observation further argues the importance of conducting different evaluations on the same tumor specimens when a study objective is to discover correlations between them, as well as when tumor microheterogeneity is of major concern.

Conclusions

The results reported here show that GBM histopathologic features correlate linearly with tumor metabolites measured with HRMAS ^1H MRS. Our spectroscopy data show a large amount of variability attributable to microheterogeneity within a single tumor and indicate strong correlations with quantified pathologic variables. Specifically, the concentrations of mobile lipids and lactate correlate linearly with the amount of necrosis, and metabolite ratios of phosphorylcholine to choline provide a measure of the proportion of tissue that is highly cellular. The variability within a single tumor region is pronounced; "adjacent" samples do not show the same percentage of histopathologic compositions. This study represents a significant advance from our previous work because it demonstrates that HRMAS does not interfere with subsequent analysis of tumor histopathologic features from the same specimen. Quantitative tumor histopathology can be performed on a specimen after it undergoes HRMAS measurement. We anticipate that quantification of tumor metabolic changes for histopathologic variability from the same tumor specimens will contribute to the establishment of MRS as a noninvasive, diagnostic procedure for brain tumors and other human malignancies. The ability to identify regions of glioma and necrosis from *in vivo* MR spectra would provide a less invasive means of clinical follow-up for low-grade gliomas and for detection of any subsequent malignant progression. Furthermore, studies of tumor metabolism will advance our understanding of the formation of neoplasia and of histopathologic heterogeneity on a biochemical level.

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