Using ex vivo proton magnetic resonance spectroscopy to reveal associations between biochemical and biological features of meningiomas

WOLFGANG K. PFISTERER, M.D.,^{1,5} RONALD A. NIEMAN, Ph.D.,⁴ ADRIENNE C. SCHECK, Ph.D.,² STEPHEN W. COONS, M.D.,³ ROBERT F. SPETZLER, M.D.,¹ AND MARK C. PREUL, M.D.¹

Divisions of ¹Neurological Surgery, ²Neuro-oncology Research, and ³Neuropathology, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix; ⁴Nuclear Magnetic Resonance Core Facility, Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona; and ⁵Neurosurgical Department, Donauspital im Sozialmedizinisches Zentrum-Ost, Vienna, Austria

Object. The goal in this study was to determine if proton (¹H) MR spectroscopy can differentiate meningioma grade and is associated with interpretations of biological behavior; the study was performed using ex vivo high-resolution spectra indicating metabolic characteristics.

Methods. Sixty-eight resected tissue samples of meningiomas were examined using ex vivo ¹H MR spectroscopy. Of these meningiomas, 46 were WHO Grade I, 14 were WHO Grade II, and 8 were WHO Grade III. Fifty-nine were primary meningiomas and 9 were recurrences. Invasion of adjacent tissue (dura mater, bone, venous sinus, brain) was found in 32 cases. Thirty-nine meningiomas did not rapidly recur (as defined by expansion on MR imaging within a 5-year follow-up period), whereas rapid recurrence was confirmed in 24 meningiomas, and follow-up status was unknown in 5 cases.

Results. The absolute concentrations of total alanine and creatine were decreased in high-grade compared with low-grade meningiomas, as was the ratio of glycine to alanine (all $p < 0.05$). Additionally, alanine and the glycine/alanine ratio distinguished between primary and recurrent meningiomas (all $p < 0.05$). Finally, the absolute concentrations of alanine and creatine, and the glycine/alanine and choline/glutamate ratios were associated with rapid recurrence ($p < 0.05$).

Conclusions. These data indicate that meningioma tissue can be characterized by metabolic parameters that are not typically identified by histopathological analysis alone. Creatine, glycine, and alanine may be used as markers of meningioma grade, recurrence, and the likelihood of rapid recurrence. These data validate a previous study of a separate group of Grade I meningiomas. (DOI: 10.3171/2009.11.FOCUS09216)

KEY WORDS • brain neoplasm • meningioma • tumor metabolism • proton magnetic resonance spectroscopy • creatine • glycine • alanine

MENINGIOMAS, despite categorization as benign lesions, may behave aggressively (that is, at rates as high as 20%), even those of low histological grade.^{37,38} In a previous study on Grade I meningioma tumor tissue in which genetic characteristics were correlated with data from ex vivo ¹H MR spectroscopy on the same tumor tissue, an identifiable subset of tumor metabolic characteristics was associated with increased aggression, even within only Grade I tumors.⁴² Meningiomas may grow quickly, invade adjacent brain, recur rapidly, and ultimately lead to decreased patient survival and quality of life.

Despite the recently revised 2000 WHO grading scheme for meningiomas, in which overall behavior correlates well with grade, aggressive behavior is sometimes difficult to predict.²⁹ Complicating the follow-up inter-

pretation of prognosis, recurrence, and grade is the fact that resection has an overwhelming influence on outcome, even for high-grade tumors. Given discrepancies between the clinical behavior, histological grading criteria, and biological makeup of these tumors, the need exists both for adjunctive tools for the improved diagnosis and prognostication of outcomes in meningiomas and for a better understanding of the biological pathogenesis of these tumors.

According to current WHO histological grading criteria, intracranial meningiomas are classified as follows: between 85 and 94% are benign (Grade I), with a benign clinical course and a 7–20% recurrence rate. Between 5 and 11% of meningiomas are atypical (Grade II), with a more aggressive clinical course and a 29–40% rate of recurrence. Between 1 and 3% of meningiomas are anaplastic (Grade III), with a very aggressive clinical course, invasion, recurrence, and metastases.²⁹ Patients with Grade III tumors have a median survival after diagnosis of ~ 1.5

Abbreviations used in this paper: ¹H = proton; TSP = sodium 3-(trimethylsilyl)propionate-2,2,3,3-d₄.

years, and a 5-year mortality rate of 68%.³⁷ Usually, clinically aggressive behavior includes arachnoid penetration, bone invasion and destruction, rapid regrowth of a residual tumor, or recurrence of a “totally resected” tumor,^{26,37} yet at surgery even fully benign meningiomas may be observed to possess many of these characteristics.

Clinical and pathological findings and resection assessment remain the standard for differentiating between meningioma grades and predicting aggressive tumor behavior, although with a high degree of inaccuracy. Our previous meningioma metabolic studies elaborated on characteristics noted in clinical and pathological studies of meningiomas.^{1,18,19,24,29,33,37,38,42,47,53} Variability in recurrence within resection (Simpson) grades suggests that subsets of these tumors exist in which other factors have a greater influence on tumor recurrence than extent of resection.

Various in vitro techniques have been used in the search for prognostic markers in meningiomas. These include immunohistochemical analyses,^{3,4,13,34,39} cytogenetic and molecular genetic analyses,^{2,5,6,17,20,28,30,41,52,59,60} ¹H MR spectroscopy analyses,^{22,23,27,36,44} and gene expression analyses.^{11,58} Overall, although many of these studies have explored biological differences between meningioma grades and/or survival rates, they have not examined biological parameters against a strictly defined clinical behavior such as rapid recurrence within a specific time period.

Histological analysis alone is no longer sufficient to characterize tumors, and it is now recognized that simply describing genetic variations in tissue or disease may not indicate new avenues for treatment (the ultimate goal). The science of proteomics, or really our understanding of metabolism, may provide the link necessary to exploit genomics. The ¹H MR spectroscopy modality has specific diagnostic potential because it can be used to measure the concentrations of major metabolites in brain tumors in vivo,^{9,10,12,16,35,43–45,48,54,56} providing a noninvasive quantitative measure of metabolic parameters that can be correlated to clinical parameters. These metabolic features are the proton-containing moieties, which may be part of full-scale proteins or which are proteinogenic, such as the amino acids glycine or alanine.

Such metabolic features can also be generated ex vivo in brain tumor extracts.^{7,21,22,25,27,36,55} Analyses of tumor extract metabolite spectra ex vivo have enhanced the ability to interpret in vivo data, not only by allowing extracts to be studied at a higher magnetic field strength, giving greater spectral dispersion than in vivo spectra, but they have also allowed for an improved understanding of how variations in tumor metabolism contribute to variations in phenotype.^{7,8,15,56,57} Creatine, glycine, alanine, lactate, choline, glutamine, glutamate, and the glutamine/glutamate complex are the metabolites most often cited as being useful in differentiating meningiomas from other tumors and from normal brain, and they are also the metabolites that are most consistently distinct and well resolved to allow for definitive quantitation.^{31,44,45}

We studied the biochemical profiles of a series of 68 clinically and histologically diverse meningiomas. We have previously shown that ¹H MR spectroscopy stud-

ies can indicate metabolic tumor features associated with clinical aggression or status of recurrence, and with chromosomal profiles, even within a group of so-called benign tumors.^{40,42} In this study, we chose to focus on metabolic features based on ¹H MR spectroscopy differences between pathological grades of meningiomas with a 5-year follow-up period. In addition, this study did not include the group of tumors from our previous study.⁴² Thus, in many respects, this study also provided validation between 2 large groups of samples of meningioma tissue studied for biochemical characteristics, which to date has not been accomplished.

Methods

Patients and Tumor Specimens

Tumor samples were collected in 68 patients (41 women [60%] and 27 men [40%]), whose ages ranged between 29 and 84 years, with a mean of 55 years, who underwent resection of their tumors between 1986 and 2005 at the Barrow Neurological Institute and the Neurosurgical Department of the Donauspital, Sozialmedizinisches Zentrum-Ost. Male patients were somewhat younger than females (mean 57 ± 10 years for men, compared with 59 ± 14 years for women; unless otherwise specified, values are expressed as the mean $\pm$ SE). The surgeon recorded the existence of dural, venous sinus, and/or bone invasion as well as brain invasion, and the extent of tumor resection according to the Simpson grading scale.⁵³ Tumor tissues were immediately snap frozen in liquid nitrogen. The histopathological section of each collected specimen was reviewed to confirm that tissue used for extracts was appropriate. Corresponding paraffin-embedded surgically obtained neuropathology samples were also reviewed to assign grades in accordance with the 2000 WHO criteria.²⁹ Clinical follow-up ranged from 12 to 108 months, with a mean of 57 ± 15 months. The meningiomas for which an MR imaging–confirmed recurrence was found within 5 years were considered to have rapidly recurred. The meningiomas for which follow-up data were available over a 5-year period and for which no recurrence was found were considered not to have rapidly recurred. None of the tissue samples we examined had been exposed to radiation therapy.

Ex Vivo ¹H MR Spectroscopy

Frozen tumor specimens were taken from the same sections in which histological characterization was performed. Preparation of perchloric acid extracts was performed according to the protocol used by Lehnhardt et al.²⁷ The ¹H MR spectroscopy was performed at the Nuclear Magnetic Resonance Facility at Arizona State University. The perchloric acid extracts were redissolved in 0.6 ml deuterium oxide containing 0.05 wt % TSP, cooled to 0°C for 10 minutes, centrifuged to remove any particulates, and transferred into a 5-mm NMR tube. Spectra were acquired at 11.4 T (500 MHz for ¹H) with a Varian Inova spectrometer, using 90° single-pulse excitation, 256 transients, a sweep width of 8000 Hz, 1-second weak preirradiation to reduce residual HDO signal, and

Correlation of ¹H MR spectroscopy with meningioma behavior

TABLE 1: Invasion type in relation to recurrence at follow-up in 63 patients with meningioma

Type of Invasion	Recurrence at Follow-Up		Total
	Yes	No	
none	0	30	30
dural only	2	3	5
bone	5	3	8
sinus	5	3	8
bone & sinus	5	0	5
brain	2	0	2
brain & sinus	2	0	2
brain & bone	3	0	3
total	24*	39	63

* Invasion was associated with rapid recurrence ($p = 0.01$).

TABLE 2: Resection grade in relation to recurrence at follow-up in 63 patients with meningioma

Resection Grade	Recurrence at Follow-Up		Total
	Yes	No	
1			
invasive	3	3	6
noninvasive	0	21	21
2			
invasive	3	6	9
noninvasive	0	9	9
3			
invasive	18	0	18
noninvasive	0	0	0
total	24*	39	63

* Resection grade (Simpson Grades 1–3) was associated with recurrence ($p = 0.001$).

Results

Clinical Parameters

The most common location was frontotemporal skull base (23 lesions [34%]), followed by convexity (19 [28%]), falx (14 [21%]), and tentorium/posterior fossa (12 [17%]). The extent of resection in 32 tumors (47%) was Simpson Grade 1, in 18 (26%) it was Grade 2, and in 18 (26%) it was Grade 3. According to WHO criteria, tumors were classified as Grade I in 46 cases (68%), Grade II in 14 (21%), and Grade III in 8 (12%). Fifty-nine meningiomas (87%) were primary and 9 (13%) were recurrent tumors. Among 9 histopathological subtypes, 22 transitional (32%), 12 meningothelial (17%), 9 fibrous (13%), 8 atypical (12%), 5 anaplastic (7%), 4 angiomatous (6%), 3 psammomatous (5%), 3 papillary (5%), and 2 fibroplastic (3%) lesions were found. Invasion of adjacent tissue (dura mater, bone, venous sinus, brain) was found in 32 cases (47%). In 4 cases without and in 1 case with bone invasion, clinical follow-up was not available. Invasion by location and by sex was not statistically significant. For greater power of statistical assessment, Grade II tumors were combined with Grade III lesions.

Among the 63 individuals for whom follow-up was available, 7 of the 43 Grade I meningiomas and 17 of the 20 Grades II (9 of 12) and III (8 of 8) meningiomas recurred. This was statistically significant ($p = 0.001$, Fisher exact test). The relationship between invasion and rapid recurrence was significant ($p = 0.01$, Fisher exact test; Table 1), as was the association between resection grade and recurrence ($p = 0.001$, Fisher exact test; Table 2). Only 6 of the 48 patients in whom total tumor resection (Simpson Grade 1 or 2) was achieved experienced recurrence of tumor, compared with all of the 18 patients who had subtotal resection (Simpson Grade 3).

a 3.39-second total recycle time, adequate to give full relaxation of all resonances. Spectra were analyzed using commercially available software (MestRe-C NMR Data Processing Package for Windows, Unidade de Resonância Magnética). Spectra were Fourier transformed, phase corrected and polynomial baseline corrected, and the appropriate peaks were picked by chemical shift and were integrated.¹⁴ The concentration of each metabolite was measured by comparing the intensity of the identified compound with that of the TSP methyl residues. Assignments were confirmed from COSY and HMQC spectra. The spectra of several samples were obtained over the course of 18 hours to control for possible sample degradation, and showed no changes in chemical shifts or integrated intensities.

The ¹H MR spectroscopy modality was used to measure the absolute concentrations and ratios of creatine, glycine, alanine, lactate, choline, glutamine, glutamate, and the glutamine/glutamate complex. Metabolites were first examined according to histological grade to look for biochemical alterations that might be correlated with phenotypes as well as specific metabolites that might be used diagnostically in conjunction with standard histological criteria to confirm grade, to distinguish between primary and recurrent tumor, and between invasive or noninvasive behavior.

Data Analysis and Statistical Methods

Summary statistics were completed for several variables for group comparison (ClinMetrics, Inc.). Categorical variables (histological grade, patient sex, primary or recurrent tumor, histopathological subtype, invasion, resection grade, and recurrence on follow-up) summarized by frequencies and percentages were compared using chi-square or Fisher exact tests as appropriate. Continuous variables (patient age, metabolite concentrations, metabolite ratios) were computed using ANOVA. All statistical tests were conducted using a significance level of 0.05.

Fig. 1. **Left:** A ^1H MR spectrum from a Grade I meningioma tissue extract shows many metabolites, among which are the prominent choline peak at 3.2 ppm, the alanine peak at 1.4 ppm, and a robust lactate peak at 1.3 ppm. **Right:** In comparison, a spectrum from a recurrent tumor tissue extract shows many of the peaks to be reduced, but there is prominent reduction of choline and alanine. Ala = alanine; Cho = choline; Cre = creatine; Gln = glutamine; Glu = glutamate; Gly = glycine; Lac = lactate.

Ex Vivo ^1H MR Spectroscopy

Representative spectra from 2 meningiomas are portrayed in Fig. 1. The mean absolute concentrations of metabolites for Grade I versus Grades II and III meningiomas are shown in Fig. 2. Although several metabolites were selected for analysis and several trends are apparent, only a few metabolite concentrations and ratios were found to correlate significantly with clinical parameters. However, when comparing WHO Grade I (46 lesions) against Grades II and III (22), the mean metabolite values for creatine and alanine are found to be significant between groups ($p < 0.05$). The mean creatine value for Grade I was 183 ± 32 μmol per 100 g wet weight of tissue compared with the other group (Grades II and III; mean 79 ± 21). The mean alanine value was lower for meningiomas categorized as Grades II and III (mean 245 ± 42) compared with Grade I tumors (mean 393 ± 43 ; $p < 0.05$). In addition, the metabolite ratio of glycine to alanine correlated significantly with tumor grade ($p = 0.002$). The mean glycine/alanine value for Grade I was 0.96 ± 0.31 , compared with the mean of 1.8 ± 0.37 for Grades II and III. Hence, alanine and creatine concentrations are lower, whereas glycine/alanine is higher in histologically aggressive meningiomas.

Neither descriptive and demographic variables nor

Fig. 2. Bar graph showing values (mean $\pm$ SE) for various prominent metabolites in the ^1H MR spectrum of meningioma tissue extracts (* $p = 0.002$).

any other metabolite concentrations or ratios correlated significantly with grade. Neither metabolites nor metabolite ratios correlated with histopathological subtype.

Only for alanine were there significant associations between metabolites and primary versus recurrent tumors ($p < 0.05$). Metabolites also may not predict primary/recurrent tumors. There were significant differences observed between primary and recurrent tumors for metabolite ratios of glycine/alanine ($p < 0.001$) and choline/glutamate ($p < 0.05$) (Fig. 3).

Individual metabolites are not associated with presence or absence of invasion. However, several metabolite ratios are associated with invasion: the ratio of lactate to glutamine/glutamate complex showed the highest significance ($p < 0.001$), with glycine/alanine and the ratio of choline to glutamine/glutamate complex following (both $p < 0.05$) (Fig. 4).

Finally, metabolites were examined against the parameter of recurrence within the follow-up period for tumors in which follow-up data were available (63 lesions). Of all metabolite concentrations and ratios studied, creatine and alanine were associated with tumor recurrence. The mean creatine and alanine concentrations were found to be significantly lower in tumors that rapidly recurred

Fig. 3. Bar graph showing selected metabolite ratio values for glycine/alanine and choline/glutamate for 68 primary and recurrent meningiomas (** $p < 0.001$, * $p < 0.05$).

FIG. 4. Bar graph showing metabolite ratio values for 68 meningiomas interpreted as showing invasion (* $p < 0.05$, ** $p < 0.001$).

compared with those that did not (both $p < 0.001$) (Fig. 5). The glycine/alanine metabolite ratio was also significantly higher in tumors with invasion than in those without ($p = 0.02$). The mean glycine/alanine ratio for patients experiencing recurrent tumors at follow-up was 1.53 ± 0.43 , compared with the mean for those who did not have recurrence (1.06 ± 0.14).

Discussion

Accurate diagnosis and prognostication of meningiomas is limited by several factors in the clinic and in the laboratory. From a pathology perspective, these limitations include basing diagnosis on morphological changes downstream of causative molecular events. In the laboratory, studies are often limited by an ambiguous definition of an aggressive meningioma. Often studies either poorly define the phenotypic components of an aggressive meningioma or simply defer to WHO grading. When they base aggression solely on WHO grade, they seek correlations between biological and pathological data instead of correlations between biological and phenotypic data (clinical outcome). We have evaluated the ability of ^1H MR spectroscopy to examine proton-containing proteins and other metabolites to enhance the diagnosis and prognostication of these tumors based on ex vivo examinations of tissue samples. This was accomplished as follows: 1) we have compared ^1H MR spectroscopy to clinical and pathological analysis techniques typically used in the diagnosis and prognostication of meningiomas; and 2) we have evaluated ^1H MR spectroscopy in clearly demarcated clinically aggressive versus clinically benign WHO Grade I meningiomas, narrowly defining clinically aggressive meningiomas in this study as those that recurred on follow-up within 5 years of resection.

Previous work in our laboratory has shown that analyses of chromosomal aberrations and analyses of metabolites yield predictors of clinical aggression that provide vital adjuncts to clinical and pathological analyses within a single grade of meningioma.^{40,42} Among all clinical and histopathological findings, resection grade and MIB-1 labeling index have been predictors of recurrence.⁴⁰

FIG. 5. Bar graph showing mean creatine and alanine values (mean $\pm$ SE) with complete follow-up data for 63 meningiomas that rapidly recurred, compared with those that did not recur (** $p < 0.001$).

Tumors are typically characterized by histologically scanning tissue for the most malignant region. Such interpretations have a significant impact on decisions regarding prognosis and choice of treatment. This examination principle also has merits for a biochemical and molecular approach to tumor behavioral assessment, and is becoming more important in more accurately predicting tumor behavior. Our previous studies of meningiomas show the impact of a regional metabolic and genetic survey of tissue.^{40–42} Glycine, alanine, choline, creatine, glutamine, and glutamate were found to play defining roles in the recurring phenotype. In this study we expanded the model we used for examination of “benign” meningiomas into a larger set of lesions that spanned multiple grades in addition to several clinical parameters of aggression. This study was composed of a separate group of tumors from our study of benign meningiomas,⁴² although many of the general metabolic characteristics we identified with recurrence, and so on, in our previous study held true in this study. Therefore, this series serves in part as a validation study, which is rare for MR spectroscopy studies of tumors.

Important Metabolites

Creatine. Compared with normal brain, the peak from creatine (creatine + phosphocreatine) is typically nearly absent in meningiomas, especially in comparison with levels seen in more malignant tumors such as medulloblastoma and glioblastoma.²² In this study, creatine was lowest in rapidly recurring tumors. In our previous work, creatine was also the metabolite whose absolute quantity was closest to approaching a significant association with rapid recurrence, being lower in those meningiomas that rapidly recurred.⁴² Creatine is usually used as an indicator of energy metabolism in the cell, although its exact function in many tumors is unknown. However, like high-grade gliomas, which show a lower signal from creatine compared with low-grade gliomas, recurrent meningiomas, which are likely to be more ag-

gressive, show lower signals from creatine compared with Grade I meningiomas.^{44–46}

Glycine. This metabolite has been found to be relatively low in normal brain tissue, but is elevated in tumors such as medulloblastoma, ependymoma, and glioblastoma.²² One study performed by magic angle spinning ¹H MR spectroscopy of 6 intact brain tissue specimens showed glycine to be absent in meningiomas or present at low levels.⁷ However, similar to our findings, elevated levels of glycine are clearly detected in extracts of meningioma tissue by ¹H MR spectroscopy.²² Neither study differentiated between meningioma types. In our study, glycine appears with high concentrations even in low-grade and clinically benign meningiomas. The high variability in levels of glycine present in some of the tumors may account for the discrepancies in earlier reports, and suggests the existence of more complex subsets within the pathological delineation of Grade I meningiomas.^{21–23,36} Glycine appears to be a metabolite worthy of future attention.

Alanine. Alanine has generally been found to be elevated in meningiomas relative to other tumors and normal brain,^{32,44,45} and appears in our study at concentrations comparable to literature values. In this study, alanine and glycine/alanine levels correlated with histological grade and with primary/recurrent status of the samples. The alanine concentration was lower in those meningiomas that rapidly recurred and in Grade III tumors. Alanine has been used as a nearly specific marker to distinguish meningiomas from gliomas and metastases by using ¹H MR spectroscopy.⁴⁴ Why meningiomas display a prominent peak from alanine is unknown, but we have determined that this metabolite is seen in quantity in extracts of dural tissue. Alanine may be produced by meningiomas in relatively larger quantities compared with other tumors, or it may be a by-product and collect within the tissue. Interestingly, alanine appears to be a “normal” part of the meningioma metabolism. As confirmed in this study, Grade I meningiomas show increased resonances for alanine compared with higher-grade meningiomas, and compared with recurrent meningiomas.⁴² Thus, as the meningioma becomes more aggressive, it loses its “normal” metabolic process for alanine. Whether alanine collects as a by-product or is specifically produced by the cells, interruption of alanine metabolism may represent a novel, convenient, specific target for developing therapy against meningioma growth.

All of the aforementioned metabolites have been shown to play various roles in cellular metabolism related to oncogenesis and progression; these alterations may be causative or constitutive of the clinically aggressive phenotype. Other metabolites require critical analysis, such as the glutamine/glutamate complex. Beyond identification of metabolites and their patterns, defining their roles and associations to clinical, genetic, and proteomic data is crucial toward a functional understanding of tumors. Few studies have accomplished this goal.⁴² Past studies of meningiomas have shown the ability of ¹H MR spectroscopy to characterize creatine and alanine levels in vivo. Although glycine has not been as well characterized by ¹H MR spectroscopy, our results, for example, suggest

that additional effort is warranted to resolve glycine in vivo to allow for further definition of aggressive subtypes of these tumors.^{16,44,45}

Recent studies of meningioma progression suggest that complex alterations seen in malignant tumors are already apparent in the early benign stages of Grade I tumors, characteristic of aggressive behavior.^{2,42} The presence of certain metabolic aberrations may indicate a more aggressive tumor, but further work needs to be done to define very specific biological subtypes of these tumors, in addition to more thorough examination of separate groups of Grade II and Grade III meningiomas. Standardization of MR spectroscopy analysis techniques will be necessary, yet there will also be influence from sampling bias of the tumor. High-throughput profiling techniques such as gene expression microarray, epigenetic screening, or proteomics assessments may allow for more robust definitions of phenotypic subtypes and correlate with the use of ¹H MR spectroscopy for brain tumor classification and therapy planning.⁵¹ Furthermore, it is not known how radiation affects the metabolic behavior of meningiomas. Perhaps somewhat surprisingly, none of the high-grade recurrent tumors had been exposed to radiation therapy.

Conclusions

Because chemical changes precede structural changes for any cell population or tissue, ¹H MR spectroscopy may provide a means of biochemical assessment for early detection of more aggressive tumors, or of those that may be in the process of becoming more aggressive. An extrapolation from this work is the use of ex vivo or in vivo MR spectroscopy to monitor brain tumor metabolism under treatment and to observe shifts in tumor activity with progression or regression.^{49,50} Meningiomas, however, will be challenging to assess with regard to how and whether such metabolic information will affect treatment planning. These tumors may be aggressive or slow growing, may become quiescent, and are significantly affected by extent of resection and by sampling, and by interpretation of variability and bias with regard to “recurrence” and “invasion” of the arachnoidal membrane. Future work will mandate the application of similar high-resolution MR spectroscopy analyses to a larger number of tumor samples that can guide further exploration of the diagnostic potential of these metabolites in vivo.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper. This work was supported by funds from the Newsome Endowed Chair of Neurosurgery Research at the Barrow Neurological Institute, the Barrow Women’s Foundation, and the Barrow Neurological Foundation.

Author contributions to the study and manuscript preparation include the following. Conception and design: MC Preul, WK Pfisterer, RF Spetzler. Acquisition of data: WK Pfisterer, RA Nieman, AC Scheck, SW Coons. Analysis and interpretation of data: MC Preul, WK Pfisterer, RA Nieman, AC Scheck, SW Coons. Drafting the article: WK Pfisterer, RA Nieman, SW Coons. Critically revising the article: MC Preul, WK Pfisterer, RA Nieman,

AC Scheck. Reviewed final version of the manuscript and approved it for submission: MC Preul, WK Pfisterer. Statistical analysis: WK Pfisterer, RA Nieman. Administrative/technical/material support: AC Scheck, RF Spetzler. Study supervision: MC Preul.

References

- Adegbite AB, Khan MI, Paine KW, Tan LK: The recurrence of intracranial meningiomas after surgical treatment. **J Neurosurg** **58**:51–56, 1983
- Al-Mefty O, Kadri PA, Pravdenkova S, Sawyer JR, Stangeby C, Husain M: Malignant progression in meningioma: documentation of a series and analysis of cytogenetic findings. **J Neurosurg** **101**:210–218, 2004
- Bitzer M, Opitz H, Popp J, Morgalla M, Gruber A, Heiss E, et al: Angiogenesis and brain oedema in intracranial meningiomas: influence of vascular endothelial growth factor. **Acta Neurochir (Wien)** **140**:333–340, 1998
- Bouillot P, Pellissier JF, Devictor B, Graziani N, Bianco N, Grisoli F, et al: Quantitative imaging of estrogen and progesterone receptors, estrogen-regulated protein, and growth fraction: immunocytochemical assays in 52 meningiomas. Correlation with clinical and morphological data. **J Neurosurg** **81**:765–773, 1994
- Cai DX, Banerjee R, Scheithauer BW, Lohse CM, Kleinschmidt-Demasters BK, Perry A: Chromosome 1p and 14q FISH analysis in clinicopathologic subsets of meningioma: diagnostic and prognostic implications. **J Neuropathol Exp Neurol** **60**:628–636, 2001
- Cerdá-Nicolás M, López-Ginés C, Pérez-Bacete M, Barcia-Salorio JL, Llombart-Bosch A: Histopathological and cytogenetic findings in benign, atypical and anaplastic human meningiomas: a study of 60 tumors. **J Neurooncol** **47**:99–108, 2000
- Cheng LL, Chang IW, Louis DN, Gonzalez RG: Correlation of high-resolution magic angle spinning proton magnetic resonance spectroscopy with histopathology of intact human brain tumor specimens. **Cancer Res** **58**:1825–1832, 1998
- Czernicki Z, Horsztynski D, Jankowski W, Grieb P, Walecki J: Malignancy of brain tumors evaluated by proton magnetic resonance spectroscopy (1H-MRS) in vitro. **Acta Neurochir Suppl** **76**:17–20, 2000
- De Edelenyi FS, Rubin C, Estève F, Grand S, Décorps M, Lefournier V, et al: A new approach for analyzing proton magnetic resonance spectroscopic images of brain tumors: nosologic images. **Nat Med** **6**:1287–1289, 2000
- De Stefano N, Caramanos Z, Preul MC, Francis G, Antel JP, Arnold DL: In vivo differentiation of astrocytic brain tumors and isolated demyelinating lesions of the type seen in multiple sclerosis using 1H magnetic resonance spectroscopic imaging. **Ann Neurol** **44**:273–278, 1998
- Fathallah-Shaykh HM, He B, Zhao LJ, Engelhard HH, Cerullo L, Lichter T, et al: Genomic expression discovery predicts pathways and opposing functions behind phenotypes. **J Biol Chem** **278**:23830–23833, 2003
- Fountas KN, Kapsalaki EZ, Gotsis SD, Kapsalakis JZ, Smisson HF III, Johnston KW, et al: In vivo proton magnetic resonance spectroscopy of brain tumors. **Stereotact Funct Neurosurg** **74**:83–94, 2000
- Goldman CK, Bharara S, Palmer CA, Vitek J, Tsai JC, Weiss HL, et al: Brain edema in meningiomas is associated with increased vascular endothelial growth factor expression. **Neurosurgery** **40**:1269–1277, 1997
- Govindaraju V, Basus VJ, Matson GB, Maudsley AA: Measurement of chemical shifts and coupling constants for glutamate and glutamine. **Magn Reson Med** **39**:1011–1013, 1998
- Griffin JL: Metabolic profiles to define the genome: can we hear the phenotypes? **Philos Trans R Soc Lond B Biol Sci** **359**:857–871, 2004
- Howe FA, Barton SJ, Cudlip SA, Stubbs M, Saunders DE, Murphy M, et al: Metabolic profiles of human brain tumors using quantitative in vivo 1H magnetic resonance spectroscopy. **Magn Reson Med** **49**:223–232, 2003
- Ishino S, Hashimoto N, Fushiki S, Date K, Mori T, Fujimoto M, et al: Loss of material from chromosome arm 1p during malignant progression of meningioma revealed by fluorescent in situ hybridization. **Cancer** **83**:360–366, 1998
- Jung HW, Yoo H, Paek SH, Choi KS: Long-term outcome and growth rate of subtotaly resected petroclival meningiomas: experience with 38 cases. **Neurosurgery** **46**:567–575, 2000
- Kallio M, Sankila R, Hakulinen T, Jääskeläinen J: Factors affecting operative and excess long-term mortality in 935 patients with intracranial meningioma. **Neurosurgery** **31**:2–12, 1992
- Ketter R, Henn W, Niedermayer I, Steilen-Gimbel H, König J, Zang KD, et al: Predictive value of progression-associated chromosomal aberrations for the prognosis of meningiomas: a retrospective study of 198 cases. **J Neurosurg** **95**:601–607, 2001
- Kinoshita Y, Kajiwarra H, Yokota A, Koga Y: Proton magnetic resonance spectroscopy of astrocytic tumors: an in vitro study. **Neurol Med Chir (Tokyo)** **33**:350–359, 1993
- Kinoshita Y, Kajiwarra H, Yokota A, Koga Y: Proton magnetic resonance spectroscopy of brain tumors: an in vitro study. **Neurosurgery** **35**:606–614, 1994
- Kinoshita Y, Yokota A: Absolute concentrations of metabolites in human brain tumors using in vitro proton magnetic resonance spectroscopy. **NMR Biomed** **10**:2–12, 1997
- Kleinpeter G, Böck F: Invasion of the cavernous sinus by medial sphenoid meningioma—“radical” surgery and recurrence. **Acta Neurochir (Wien)** **103**:87–91, 1990
- Kuesel AC, Sutherland GR, Halliday W, Smith IC: 1H MRS of high grade astrocytomas: mobile lipid accumulation in necrotic tissue. **NMR Biomed** **7**:149–155, 1994
- Kujas M: Meningioma. **Curr Opin Neurol** **6**:882–887, 1993
- Lehnhardt FG, Röhn G, Ernestus RI, Grüne M, Hoehn M: 1H- and (31)P-MR spectroscopy of primary and recurrent human brain tumors in vitro: malignancy-characteristic profiles of water soluble and lipophilic spectral components. **NMR Biomed** **14**:307–317, 2001
- López-Ginés C, Cerdá-Nicolás M, Gil-Benso R, Barcia-Salorio JL, Llombart-Bosch A: Loss of 1p in recurrent meningiomas: a comparative study in successive recurrences by cytogenetics and fluorescence in situ hybridization. **Cancer Genet Cytogenet** **125**:119–124, 2001
- Louis DN, Scheithauer BW, Budka H, von Deimling A, Kepes JJ: Meningiomas, in Kleihues P, Cavenee WK (eds): **Pathology and Genetics of Tumours of the Nervous System: World Health Organisation Classification of Tumours**. Lyon: IARC Press, 2000, pp 176–184
- Maíllo A, Díaz P, Sayagués JM, Blanco A, Tabernero MD, Ciudad J, et al: Gains of chromosome 22 by fluorescence in situ hybridization in the context of an hyperdiploid karyotype are associated with aggressive clinical features in meningioma patients. **Cancer** **92**:377–385, 2001
- Majós C, Alonso J, Aguilera C, Serrallonga M, Pérez-Martín J, Acebes JJ, et al: Proton magnetic resonance spectroscopy ((1)H MRS) of human brain tumours: assessment of differences between tumour types and its applicability in brain tumour categorization. **Eur Radiol** **13**:582–591, 2003
- Manton DJ, Lowry M, Blackband SJ, Horsman A: Determination of proton metabolite concentrations and relaxation parameters in normal human brain and intracranial tumours. **NMR Biomed** **8**:104–112, 1995
- Marks SM, Whitwell HL, Lye RH: Recurrence of meningiomas after operation. **Surg Neurol** **25**:436–440, 1986
- Nagashima G, Aoyagi M, Wakimoto H, Tamaki M, Ohno K, Hirakawa K: Immunohistochemical detection of progesterone

- receptors and the correlation with Ki-67 labeling indices in paraffin-embedded sections of meningiomas. **Neurosurgery** **37**:478–483, 1995
35. Ott D, Hennig J, Ernst T: Human brain tumors: assessment with in vivo proton MR spectroscopy. **Radiology** **186**:745–752, 1993
 36. Peeling J, Sutherland G: High-resolution ¹H NMR spectroscopy studies of extracts of human cerebral neoplasms. **Magn Reson Med** **24**:123–136, 1992
 37. Perry A, Scheithauer BW, Stafford SL, Lohse CM, Wollan PC: “Malignancy” in meningiomas: a clinicopathologic study of 116 patients, with grading implications. **Cancer** **85**:2046–2056, 1999
 38. Perry A, Stafford SL, Scheithauer BW, Suman VJ, Lohse CM: Meningioma grading: an analysis of histologic parameters. **Am J Surg Pathol** **21**:1455–1465, 1997
 39. Perry A, Stafford SL, Scheithauer BW, Suman VJ, Lohse CM: The prognostic significance of MIB-1, p53, and DNA flow cytometry in completely resected primary meningiomas. **Cancer** **82**:2262–2269, 1998
 40. Pfisterer WK, Coons SW, Aboul-Enein F, Hendricks WP, Scheck AC, Preul MC: Implicating chromosomal aberrations with meningioma growth and recurrence: results from FISH and MIB-I analysis of grades I and II meningioma tissue. **J Neurooncol** **87**:43–50, 2008
 41. Pfisterer WK, Hank NC, Preul MC, Hendricks WP, Pueschel J, Coons SW, et al: Diagnostic and prognostic significance of genetic regional heterogeneity in meningiomas. **Neuro-oncol** **6**:290–299, 2004
 42. Pfisterer WK, Hendricks WP, Scheck AC, Nieman RA, Birkner TH, Krampla WW, et al: Fluorescent in situ hybridization and ex vivo ¹H magnetic resonance spectroscopic examinations of meningioma tumor tissue: is it possible to identify a clinically-aggressive subset of benign meningiomas? **Neurosurgery** **61**:1048–1061, 2007
 43. Poptani H, Kaartinen J, Gupta RK, Niemitz M, Hiltunen Y, Kauppinen RA: Diagnostic assessment of brain tumours and non-neoplastic brain disorders in vivo using proton nuclear magnetic resonance spectroscopy and artificial neural networks. **J Cancer Res Clin Oncol** **125**:343–349, 1999
 44. Preul MC, Caramanos Z, Collins DL, Villemure JG, Leblanc R, Olivier A, et al: Accurate, noninvasive diagnosis of human brain tumors by using proton magnetic resonance spectroscopy. **Nat Med** **2**:323–325, 1996
 45. Preul MC, Caramanos Z, Leblanc R, Villemure JG, Arnold DL: Using pattern analysis of in vivo proton MRSI data to improve the diagnosis and surgical management of patients with brain tumors. **NMR Biomed** **11**:192–200, 1998
 46. Preul MC, Leblanc R, Caramanos Z, Kasrai R, Narayanan S, Arnold DL: Magnetic resonance spectroscopy guided brain tumor resection: differentiation between recurrent glioma and radiation change in two diagnostically difficult cases. **Can J Neurol Sci** **25**:13–22, 1998
 47. Puchner MJ, Fischer-Lampsatis RC, Herrmann HD, Freckmann N: Suprasellar meningiomas—neurological and visual outcome at long-term follow-up in a homogeneous series of patients treated microsurgically. **Acta Neurochir (Wien)** **140**:1231–1238, 1998
 48. Ross B, Michaelis T: Clinical applications of magnetic resonance spectroscopy. **Magn Reson Q** **10**:191–247, 1994
 49. Sankar T, Caramanos Z, Assina R, Villemure JG, Leblanc R, Langleben A, et al: Prospective serial proton MR spectroscopic assessment of response to tamoxifen for recurrent malignant glioma. **J Neurooncol** **90**:63–76, 2008
 50. Sankar T, Kuznetsov YE, Caramanos Z, Antel SB, Arnold DL, Preul MC: The metabolic epicenter of supratentorial gliomas: a ¹H-MRSI study. **Can J Neurol Sci** **36**:696–706, 2009
 51. Sanson M, Cornu P: Biology of meningiomas. **Acta Neurochir (Wien)** **142**:493–505, 2000
 52. Sayagués JM, Tabernero MD, Maillou A, Díaz P, Rasillo A, Bortoluci A, et al: Incidence of numerical chromosome aberrations in meningioma tumors as revealed by fluorescence in situ hybridization using 10 chromosome-specific probes. **Cytometry** **50**:153–159, 2002
 53. Simpson D: The recurrence of intracranial meningiomas after surgical treatment. **J Neurol Neurosurg Psychiatry** **20**:22–39, 1957
 54. Tate AR, Majós C, Moreno A, Howe FA, Griffiths JR, Arús C: Automated classification of short echo time in in vivo ¹H brain tumor spectra: a multicenter study. **Magn Reson Med** **49**:29–36, 2003
 55. Usenius JP, Kauppinen RA, Vainio PA, Hernesniemi JA, Vapalahti MP, Paljärvi LA, et al: Quantitative metabolite patterns of human brain tumors: detection by ¹H NMR spectroscopy in vivo and in vitro. **J Comput Assist Tomogr** **18**:705–713, 1994
 56. Usenius JP, Tuohimetsä S, Vainio P, Ala-Korpela M, Hiltunen Y, Kauppinen RA: Automated classification of human brain tumours by neural network analysis using in vivo ¹H magnetic resonance spectroscopic metabolite phenotypes. **Neuroreport** **7**:1597–1600, 1996
 57. Usenius JP, Vainio P, Hernesniemi J, Kauppinen RA: Choline-containing compounds in human astrocytomas studied by ¹H NMR spectroscopy in vivo and in vitro. **J Neurochem** **63**:1538–1543, 1994
 58. Watson MA, Gutmann DH, Peterson K, Chicoine MR, Kleinschmidt-DeMasters BK, Brown HG, et al: Molecular characterization of human meningiomas by gene expression profiling using high-density oligonucleotide microarrays. **Am J Pathol** **161**:665–672, 2002
 59. Yakut T, Bekar A, Doygun M, Acar H, Egeli U, Ogul E: Evaluation of relationship between chromosome 22 and p53 gene alterations and the subtype of meningiomas by the interphase-FISH technique. **Teratog Carcinog Mutagen** **22**:217–225, 2002
 60. Zang KD: Meningioma: a cytogenetic model of a complex benign human tumor, including data on 394 karyotyped cases. **Cytogenet Cell Genet** **93**:207–220, 2001

Manuscript submitted September 15, 2009.

Accepted November 25, 2009.

Address correspondence to: Mark C. Preul, M.D., Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, 350 West Thomas Road, Phoenix, Arizona 85013. email: mark.preul@chw.edu.