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. SHECK, 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 interpretation 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 years.
years, and a 5-year mortality rate of 68%. Usually, clinically aggressive behavior includes arachnoid penetration, bone invasion and destruction, rapid regrowth of a residual tumor, or recurrence of a "totally resected" tumor; 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. 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, 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. 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. 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.

We studied the biochemical profiles of a series of 68 clinically and histologically diverse meningiomas. We have previously shown that 1H MR spectroscopy studies 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. 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. 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 Donau Hospital, 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 ± 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. 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. 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. 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...
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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. 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 $^1$H 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.

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).

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

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* Resection grade (Simpson Grades 1–3) was associated with recurrence (p = 0.001).
Ex Vivo $^1$H 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 $183 \pm 32$ µmol per 100 g wet weight of tissue compared with the other group (Grades II and III; mean $79 \pm 21$). The mean alanine value was lower for meningiomas categorized as Grades II and III (mean $245 \pm 42$) compared with Grade I tumors (mean $393 \pm 43$; $p < 0.05$). 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 $0.96 \pm 0.31$, compared with the mean of $1.8 \pm 0.37$ 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 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.
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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

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 recur.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.24-46

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.23 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 menin-
giomas 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 du-
rinal 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 will be necessary, yet there will also be influence from sampling bias of the tumor. High-throughput profiling techniques such as gene expression microarray, epige-
netic 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 ra-
diation therapy.

Conclusions

Because chemical changes precede structural chang-
es 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 un-
der 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 plan-
ing. 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 diagnos-
tic 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 speci-
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Author contributions to the study and manuscript prepara-
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AC Scheck. Reviewed final version of the manuscript and approved it for submission: MC Peul, WK Pfisterer. Statistical analysis: WK Pfisterer, RA Nieman. Administrative/technical/material support: AC Scheck, RF Spetzler. Study supervision: MC Peul.

References

receptors and the correlation with Ki-67 labeling indices in paraffin-embedded sections of meningiomas. *Neurosurgery* 37:478–483, 1995

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.