Predicting Outcomes of Patients With Intracranial Meningiomas Using Molecular Markers of Hypoxia, Vascularity, and Proliferation

BACKGROUND: The natural history of surgically treated intracranial meningiomas can be quite variable. Recurrence and patient outcome cannot currently be predicted with accuracy.

OBJECTIVE: To explore the potential roles of tumor hypoxia-regulated biological markers, preoperative imaging, measures of proliferation, and angiogenesis in predicting patient outcome.

METHODS: Tissue from 263 patients (average follow-up, 75 months) was examined for molecular markers hypoxia-inducible factor-1α (HIF-1α), carbonic anhydrase-IX (CA-IX), and glucose transporter-1 (Glut-1); vascular endothelial growth factor (VEGF); proliferation (MIB-1); and microvascular density (MVD) (Factor VIII). Preoperative magnetic resonance images were also examined for tumor size and peritumoral brain edema (PTBE).

RESULTS: VEGF, HIF-1α, CA-IX, and Glut-1 are positively correlated ($P < .001–.005$). PTBE was associated with higher grade ($P = .03$), larger tumors ($P = .02$), and log of MVD ($P = .004$). Progression-free survival (PFS) was associated with higher grade ($P < .001$), subtotal resection ($P = .004$), VEGF expression ($P = .004$), and log of MIB-labeling index ($P < .001$) on pairwise comparisons. Using multivariate analysis, PFS was associated with subtotal resection (HR 2.71, $P = .027$), higher grade (HR 6.29, $P < .001$), higher VEGF expression (HR 1.52, $P = .038$), and log of MIB-labeling index (HR 1.68, $P = .005$). Shorter overall survival was associated with subtotal resection (HR 3.23, $P < .001$), higher grade (HR 4.47, $P = .001$), higher expression of HIF-1α (HR 1.56, $P < .001$), and Glut-1 (HR 1.39, $P = .02$), and log of MIB-labeling index (HR 1.87, $P < .001$) when controlled for age.

CONCLUSION: HIF, VEGF, and MIB-1 are significantly correlated with tumor recurrence. With further study, these molecular markers may be used to predict outcome for patients with intracranial meningiomas.

KEYWORDS: Hypoxia, Meningioma, Outcomes, Proliferation, Recurrence, Survival, Vascularity

The natural history of intracranial meningiomas, even benign meningiomas (World Health Organization [WHO] grade I), after complete surgical resection is quite variable. Outcome for patients with atypical/malignant (WHO grade II) or anaplastic/malignant meningiomas (WHO grade III) is even less predictable and is associated with a much higher recurrence and shorter survival times.\(^{2,4}\) Extent of resection measured by Simpson grade, proliferative index measured by MIB-1 immunohistological staining, mitotic activity, and histological grade are all used to predict meningioma tumor behavior.\(^{2,5,5.7}\) Unfortunately, these factors are not completely accurate in meningioma tumor behavior prediction, especially in long-term follow-up analysis.\(^{2,5,8,12}\)

Most tumor growth is thought to be angiogenesis dependent.\(^{5,14}\) As a tumor grows beyond 1 to 2 mm\(^3\), the growth of new capillaries is induced to
sustain delivery of nutrients to the growing tumor through peptide growth factors. One of the most studied factors is vascular endothelial growth factor (VEGF), which promotes migration, proliferation, and the formation of endothelial cells. It also enhances vascular permeability, which may aid in development of peritumoral brain edema (PTBE), a common feature of meningiomas. 

VEGF is a downstream target of hypoxia-inducible factor 1α (HIF-1α), a molecular marker of hypoxia. Hypoxia, caused by the abnormal circulatory conditions found within the tumor vascular network, appears to be a primary force in driving tumor-induced angiogenesis. Although the molecular events in this pathway, initiated with oxygen sensing and culminating in increased vessel formation, are not completely understood, HIF-1α has emerged as the master regulator of adaptive responses to hypoxia. HIF-1α and the downstream regulated targets VEGF, carbonic anhydrase IX (CA-IX), and glucose transporter-1 (Glut-1) have been evaluated as potential predictors for survival and tumor grade in astrocytic tumors as well as other tumors outside the central nervous system. In this study, we investigated the associations of 4 markers of hypoxia (HIF-1α, Glut-1, VEGF, CA-IX), cellular proliferation rates as measured by MIB-1 index, tumor vascularity as measured by microvascular density (MVD) indices, extent of surgical resection, preoperative imaging characteristics, and pathological grade with progression-free survival (PFS) and overall survival (OS) rates of adults with meningioma.

**MATERIALS AND METHODS**

**Study Participants**

Under an institutional review board-approved protocol, we collected intracranial meningioma tumor specimens over a 10-year period. Patients with neurofibromatosis II were not included in this study. None of the patients in this study underwent preoperative embolization, because this has been shown to affect hypoxia-regulated protein expression. We retrospectively reviewed information on age, sex, tumor location, extent of resection, pathology, preoperative imaging, and the length of PFS and OS. Extent of resection was determined by surgical report (most of these patients were operated on by the senior author) and postoperative magnetic resonance (MR) imaging and defined as gross total resection for tumors graded as Simpson grade 1 and 2 or subtotal resection for Simpson grades 3, 4, and 5. Histological grade was determined using the 2007 WHO classification of central nervous system tumors, and tumors were categorized as benign/WHO grade I, atypical/malignant/WHO grade II, or anaplastic/malignant/WHO grade III. A single pathologist performed all surgical grading. Tumors were then grouped as low grade (WHO grade I) or higher grade (WHO grade II or III). PFS is calculated as the time from first surgery to imaging-documented progression or recurrence, and OS as the time from first surgery to death from any cause.

Data from patients included in the OS and PFS analysis were included in evaluations until the patients reached the end point or were last evaluated. Available preoperative MR imaging studies were evaluated for presence or absence of PTBE (T2-weighted hyperintensity), average tumor volume (T1-weighted contrast-enhancing volume), average edema volume, and edema index (average edema-to-tumor ratio).

**HIF-1α, VEGF, CA-IX, and Glut-1**

HIF-1α immunohistochemistry was performed as previously described by using the Catalyzed Signal Amplification System (DAKO, Carpinteria, California) according to the manufacturer’s recommended protocol and primary antibody, H11667 (Novus Biologicals, Littleton, Colorado), at a dilution of 1:1000. VEGF, CA-IX, and Glut-1 immunohistochemistry was done by using anti-VEGF Ab-1 polyclonal antibody (1:50 dilution; Calbiochem, Cambridge, Massachusetts), anti-CA-IX goat polyclonal antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, California), or rabbit anti-Glut-1 (1:100, Santa Cruz Biotechnology), and the Vectastain ABC kit (Vector Laboratories, Burlingame, California) as previously described. Slides were counterstained with toluidine blue. Negative controls replaced the primary antibody with nonimmune serum, with all other steps performed as above. Positive controls for HIF-1α, VEGF, CA-IX, and Glut-1 were performed on paraffin-fixed sections of tumors grown in mice with the use of human U251 cell lines that were immunohistochemically positive for these proteins by using the same steps as above.

All slides were examined under 200× magnification with an Olympus BX41 microscope and scored by an investigator (R.L.J.) blinded to the patient information and tumor grade. The immunohistochemical analysis of HIF-1α, VEGF, CA-IX, and Glut-1 was scored from 0 to 4 (0, 0% to <25%; 1, 25% to <50%; 2, 50% to <75%; 3, 75% to <100%; and 4, 100%) based on the number of cells stained in a given field.

**Proliferation Index**

The proliferation index (PI) was calculated using Ki-67 (clone MIB-1, dilution 1:300) on the Ventana ES (Ventana Medical Systems) as previously described. Positive controls were performed on human thymus, which has >90% cell staining. Negative controls replaced the primary antibody with nonimmune serum. PI was calculated as previously described. In brief, pictures were taken at 400× (10 ocular × 40 objective) magnification using an Olympus Microfire camera and analyzed using Image-Pro Plus 5.0. PI was calculated as the number of MIB-1-stained cells divided by the total number of cells in the field repeated 3 times for each picture and averaged (Figure 1M,N). Analysis was duplicated by a separate researcher. This method was reproducible, as demonstrated by good interrater (p = 0.99, 95% confidence interval [CI] (0.99-1.00)) and intrarater (p = 0.96, 95% CI (0.92-0.99)) reliability in previous studies.

**MVD Index**

The slides for the MVD index analysis were prepared by using the same steps as described above for the MIB-1 analysis, except that they were pretreated with Factor VIII (rabbit polyclonal, dilution 1:100) Protease 2 (Ventana Medical Systems). Negative controls replaced the primary antibody with nonimmune serum, with all other steps performed as above.

The MVD index was calculated based on a previously published method. In brief, 3 pictures of the most vascular area of the slide were taken at 200× magnification with an Olympus Microfire camera and transferred to Photoshop CS 7 (Adobe Systems Incorporated, San Jose, California). Any positive cell that was separate from other stained cells and not contiguous or branching from other vessels was counted. The results for each slide were averaged for the resulting MVD (Figure 1G, H,K,L,OP) and divided by 0.26 mm² to normalize the size of the picture field.
**Statistical Analysis**

Pairwise comparisons of known associations between progression, death, extent of resection, and pathological grade were examined by using univariate logistic regression. The associations between HIF-1α, VEGF, CA-IX, and Glut-1, which were treated as continuous variables because the interval sizes were the same, were tested for significance by the use of linear regression and Pearson correlation. χ² and logistic regression were used to evaluate statistically significant differences in age, sex, pathological grade, and extent of resection between the groups of patients in whom radiographic information was or was not available. Log of MVD and MIB was calculated to normalize the data. In the group with available radiographic information, pairwise comparisons between presence or absence of edema and age, sex, pathological grade, VEGF, HIF-1α, CA-IX, Glut-1, log of MVD, and MIB-1-labeling index were done using univariate logistic regression. Pairwise comparisons between clinical history of seizures and these variables were done by use of univariate logistic regression. Pairwise comparisons between presence or absence of progression and these variables were done by use of univariate logistic regression and χ² or Fisher exact testing. Independent variables that were statistically significant at P ≤ .1 were entered into a multivariate stepwise backward logistic regression model with the significance levels for removal and addition to the model of P = .1 and P = .05, respectively. Univariate Cox regression was used to evaluate associations between time...
to progression and time to death for each of the variables of interest, respectively. For OS and PFS Kaplan-Meier curves, the independent variables HIF-1α, VEGF, CA-IX, and Glut-1 were divided into low (immunohistochemical score of 0-1) and high (immunohistochemical score of 2-4) expression for simplicity. Independent variables that were statistically significant at \( P \leq .01 \) were entered into a multivariate stepwise backward Cox regression model with the significance levels for removal and addition to the model of \( P = .01 \) and \( P = .05 \), respectively. Age-adjusted Kaplan-Meier survival curves are reported for OS. Statistical analyses were done with the use of Stata 8.0 software (StataCorp, College Station, Texas) using 2-sided comparisons with significance set at 0.05.

**RESULTS**

**Study Patient Profile**

We prospectively examined tumor specimens from 263 patients (median age, 56 years; range, 21-95 years; 67% female) (Table 1). Ninety percent of tumors were benign, 9% were WHO grade II, and 1% were WHO grade III. Most patients underwent gross total resection. Non-skull base tumor locations were most common, probably biased by the senior author’s surgical practice. There were 217 patients available for outcome analysis, with median follow up of 75.3 months. The most common presenting symptom was headache (50% of patients). About 14% of patients had a seizure sometime during their clinical course—most presented with seizures (13%), while the remaining 1% (3 patients) experienced seizures more than 6 months postoperatively while not on antiepileptic medication.

**Expression of Hypoxia-Regulated Markers**

Two-hundred sixty-three meningioma tissue samples were analyzed for expression of the hypoxia-regulated proteins HIF-1α, VEGF, CA-IX, and Glut-1 (Table 2, Figure 1). Grouping scores of 0 and 1 together as “low score” and 2 to 4 as “high score” seems to provide the most valid analysis. Higher-grade tumors were associated with higher scores for CA-IX (\( P < .001 \)), VEGF (\( P = .02 \)), and HIF-1α (\( P = .02 \)). As expected, because VEGF, CA-IX, and Glut-1 are regulated downstream of the HIF-1α gene, VEGF, HIF-1α, and Glut-1 are positively correlated, with \( \rho \) values ranging from 0.1976 to 0.4658. (\( P < .001 -.005 \); Table 3).

**Measures of Proliferation and Vascularity**

MIB-1 index for atypical and anaplastic meningiomas had a mean of 10.79 compared with 2.4 for benign tumors (\( P < .001 \), Figure 2A). Furthermore, 210 of 236 (89.0%) of WHO grade I meningiomas had an MIB-1 index <5%, whereas 21 of 27 (78%) of WHO grade II/III meningiomas had a labeling index >5% (\( \chi^2 = 90.88, P < .001 \), Table 4).

Average MVD scores (Figure 2B, Table 4) were not statistically different (\( P = .89 \)) when comparing lower-grade (mean, 71.5) and higher-grade (mean, 93.1) meningiomas. Interestingly, if the histological groups were analyzed for MVD scores with a breakpoint of 50, 49% of WHO grade I meningiomas had a score <50, while only 22.2% of higher-grade tumors had a score <50 (\( \chi^2 = 15.92, P < .001 \), Table 4); however, with a score of 100, no such relationship existed (\( \chi^2 = 0.03, P = .86 \), Table 4).

**Imaging Analysis**

Imaging data were available for a subset of 66 subjects who were not significantly different than the entire cohort for age, sex, grade, and extent of resection. Of the images analyzed, 64% had evidence of PTBE. The mean edema index was 1.28 ± 3.80.

On univariate analysis, PTBE was associated primarily with larger tumors (\( P < .001 \)) and log of MVD (\( P = .004 \)) but not associated with age, sex, VEGF, HIF1, CA-IX, Glut-1, or MIB-1-labeling index (Table 5). All higher-grade tumors had associated edema.

**Patient Symptoms, Tumor Characteristics, and Outcome**

Headaches as a presenting symptom, history of multiple meningiomas, radiation-induced meningiomas, familial cancer history, tumor location, and other comorbidities were not found to have any relationship to predict tumor grade or patient outcome.
Presentation with seizures was associated with higher-grade tumor and female sex (Table 5). Log of MVD and MVD alone were not associated with presentation with seizure.

### Progression-Free Survival

The median PFS (n = 182) was 68.01 months (range, 0.1-138.44 months, Figure 3A). With the use of univariate Cox regression, PFS was not associated with age, sex, presence of PTBE, MVD, HIF-1α, Glut-1, or CA-IX expression, but was associated with higher grade (P < .001, Figure 3B), subtotal resection (P = .004), higher VEGF expression (P = .004, Figure 3C), and log of MIB-labeling index (P < .001, Figure 3C) on pairwise comparisons (Tables 6 and 7). With the use of multivariate Cox regression, PFS data were predicted by higher grade (HR 6.29, P < .001) and log of MIB-labeling index (HR 1.68, P = .005), VEGF expression (HR 1.52, P = .04), and subtotal resection (HR 2.71, P < .001). Higher grade was correlated with MIB (P < .001), but these variables were not completely collinear.

### Overall Survival

The median OS (n = 205) was 75.29 months (range, 0.1-156.59 months, Figure 4A). With the use of univariate Cox regression, OS was associated with age (P < .001). Adjusting for age, OS was associated with subtotal resection (HR 3.23, P = .002), higher grade (HR 4.47, P < .001), higher HIF-1α expression (HR 1.56, P < .001, Figure 4B), higher Glut-1 expression (HR 1.39, P = .04, Figure 4C), and log of MIB-labeling index (HR 1.87, P < .001, Figure 4D). Overall survival was not associated with higher VEGF expression, higher CA-IX expression, MVD, or sex. With the use of multivariate Cox regression, overall survival remained associated with age (HR 1.04, P = .002), higher HIF-1α expression (HR 1.44, P = .002), and log of MIB-labeling index (HR 1.58, P = .007, Tables 6 and 7). Atypical pathology was correlated with MIB (P < .001), but these variables were not completely collinear.

### DISCUSSION

**Expression of Hypoxia-Regulated Markers**

Tumor histological characteristics are currently used to determine pathological grade, which in turn is used to predict expected tumor behavior; however, the biological behavior of a given meningioma cannot be accounted for by histological parameters alone. In this study, we evaluated the largest cohort of subjects undergoing surgical treatment for intracranial meningioma in which hypoxia-related biochemical markers have been evaluated. As expected, because these proteins are regulated downstream of the HIF-1α gene, VEGF, HIF, CA-IX, and Glut-1 are positively correlated. Higher-grade tumors, and

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**TABLE 2. Hypoxia-Regulated Biomarker Data for All Meningioma Tissue Samples**

<table>
<thead>
<tr>
<th>Biological Marker</th>
<th>WHO Grade I (n = 236)</th>
<th>WHO Grade II/III (n = 27)</th>
<th>All Tumors (n = 263)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Score (0-1)</td>
<td>High Score (2,3,4)</td>
<td>Low Score (0-1)</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>100 (42.4)</td>
<td>136 (57.6)</td>
<td>7 (25.9)</td>
</tr>
<tr>
<td></td>
<td>χ² = 5.7, P = .02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>66 (28.0)</td>
<td>170 (72.0)</td>
<td>4 (14.8%)</td>
</tr>
<tr>
<td></td>
<td>χ² = 8.01, P = .02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glut-1</td>
<td>107 (43.3)</td>
<td>129 (54.7)</td>
<td>11 (40.7)</td>
</tr>
<tr>
<td></td>
<td>χ² = 0.17, P = .68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA-IX</td>
<td>74 (31.4)</td>
<td>162 (68.6)</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td></td>
<td>χ²=12.06, P &lt; .001</td>
<td></td>
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</tbody>
</table>

WHO, World Health Organization; HIF-1α, hypoxia-inducible factor-1α; Glut-1, glucose transporter-1; CA-IX, carbonic anhydrase-IX; VEGF, vascular endothelial growth factor.

**TABLE 3. Correlation of Hypoxia-Regulated Biological Markers**

<table>
<thead>
<tr>
<th>Hypoxia Marker</th>
<th>HIF-1α</th>
<th>VEGF</th>
<th>Glut-1</th>
<th>CA-IX</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1α</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>r = 0.198, P = .005</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glut-1</td>
<td>r = 0.377, P &lt; .001</td>
<td>r = 0.205, P = .003</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>CA-IX</td>
<td>r = 0.343, P &lt; .001</td>
<td>r = 0.262, P &lt; .001</td>
<td>r = 0.466, P &lt; .001</td>
<td>1.0</td>
</tr>
</tbody>
</table>

HIF-1α, hypoxia-inducible factor-1α; Glut-1, glucose transporter-1; CA-IX, carbonic anhydrase-IX; VEGF, vascular endothelial growth factor.
subsequently higher MIB-1 scores, were associated with higher scores for CA-IX, VEGF, and HIF-1α. They do not, however, show a similar association with MVD, a measure of angiogenesis. These markers have been previously evaluated as potential predictors for survival and tumor grade in several tumor types including gliomas. HIF-1α has emerged as the master regulator of adaptive responses to hypoxia. Among HIF-dependent genes, VEGF aids in balancing vascular supply to the metabolic needs of the tumor, acting as both a potent angiogenic molecule and a survival factor for newly formed vessels. CA-IX is a metalloenzyme that catalyzes the reversible hydration of carbon dioxide. This enzyme may play a role in tumor cell survival under hypoxic conditions by maintaining a neutral intracellular pH, contributing to acidification of the extracellular space to facilitate tumor growth and invasion. Glut-1 is the major transporter of glucose into the cell and plays important roles in cellular metabolism.

**Measures of Proliferation and Vascularity**

MIB-1-labeling indices are commonly used to quantify proliferation in many types of brain tumors. In meningiomas, this marker has generally shown a positive correlation with WHO grade and thus tumor recurrence. MVD and MIB-1-labeling index are also significantly associated. In our study, MIB-1 index for atypical and anaplastic meningiomas was significantly higher than in benign tumors. Most WHO grade I meningiomas had an MIB-1 index <5%, whereas the majority of WHO grade II/III meningiomas had a labeling index >5%. This is similar to other published series in which WHO grade I meningiomas demonstrated MIB-1 indices of 1.00% to 1.35%, whereas grade II tumors were 1.9% to 9.3%, and grade III tumors were 5.6% to 19.5%.

In astrocytomas, HIF-1α and VEGF levels are positively correlated with histological grade and MIB-1-labeling index; measures of Proliferation and Vascularity for All Meningioma Tissue Samples

**TABLE 4. Analysis of Measures of Proliferation and Vascularity for All Meningioma Tissue Samples**

<table>
<thead>
<tr>
<th>Biological Marker</th>
<th>WHO Grade I (n = 236)</th>
<th>WHO Grade II/III (n = 27)</th>
<th>All Tumors (n = 263)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MIB-1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score &lt;5</td>
<td>Score &gt;5</td>
<td>Score &lt;5</td>
<td>Score &gt;5</td>
</tr>
<tr>
<td>210 (89.0%)</td>
<td>26 (11.0%)</td>
<td>6 (22.2%)</td>
<td>21 (77.8%)</td>
</tr>
<tr>
<td>Average</td>
<td>2.23 ± 3.02</td>
<td>10.79 ± 7.74</td>
<td>3.12 ± 4.68</td>
</tr>
<tr>
<td>Median</td>
<td>1.0</td>
<td>9.32</td>
<td>1.2</td>
</tr>
</tbody>
</table>

| **MVD** | | | |
| Score <50 | Score >50 | Score <50 | Score >50 | Score <50 | Score >50 |
| 116/236 (49.0%) | 121/236 (51.0%) | 6 (22.2%) | 21 (77.8%) | 124 (47.2%) | 139 (52.8%) |
| Average | 71.47 ± 68.42 | 93.10 ± 71.01 | 74.4 ± 70.23 |
| Median | 51.21 | 69.23 | 52.6 |

*WHO, World Health Organization; MVD, microvascular density.*
Although a trend of higher Simpson grade resections was associated with presence of PTBE, the effect was not statistically different between subtotal and total resections.

Average MVD scores were not statistically different between lower-grade and higher-grade meningiomas in contrast to several studies where VEGF was upregulated and associated with higher MVD in meningiomas. VEGF and MVD were positively correlated in low-grade gliomas and predicted overall patient survival in multivariate analysis.

**Patient Imaging Analysis**

PTBE may contribute to the overall mass effect of the tumor, leading to earlier manifestation and severity of symptoms. Our findings agree with earlier reports that PTBE is found in 40% to 60% of intracranial meningiomas. We found no correlation between hypoxia-regulated protein expression and PTBE. Others have demonstrated increased VEGF expression in meningiomas with PTBE, although a recent report found that both VEGF expression and pial vascular supply were necessary for PTBE in grade I meningiomas. Although a trend toward higher HIF-1α staining and presence of PTBE was shown, it did not reach statistical significance. Similar to another study, we found that MIB-1 index does not appear to be associated with PTBE.

MVD and tumor size were predictive of PTBE in our study. Although there are several conflicting reports, most investigators have found a correlation between tumor size and PTBE. PTBE can be associated with larger tumor size, which appears to be independent of VEGF expression.

Although the exact mechanism of PTBE is unknown, the secretion of VEGF is the most accepted cause of PTBE in many tumor types, especially meningioma. This is based on evidence that VEGF enhances vascular permeability, which may aid in development of PTBE. HIF-1α is the major regulator of VEGF; both are known to be hypoxia regulated, but very little is known about the role of hypoxia in meningiomas. Nevertheless, hypoxia appears to be a primary force in driving tumor-induced angiogenesis in many other tumors.

### Progression-Free Survival

As expected, higher-grade and subtotal resections were associated with progression. Historically, these factors have been well established in the literature and in this analysis, they remain the strongest predictors of progression. Most modern patient series place the 5-year recurrence rate as approximately 40% for typical meningiomas and 50% to 80% for anaplastic meningiomas, even after complete surgical resection. As described above, MIB-1-labeling indices are commonly used to quantify proliferation in many tumors. In this and other studies, there is a positive correlation with WHO grade as well as higher probability of tumor recurrence. VEGF was associated with presence of progression, although the magnitude of the effect was less than that of higher-grade and subtotal resections. The findings reported in the literature are somewhat mixed, with some studies suggesting that higher VEGF expression is independently associated with PFS, whereas others have found no such association between VEGF and pathological grade in meningiomas. Complete surgical resection is the standard of care whenever possible, especially after evidence of tumor growth on serial imaging examination in symptomatic patients. It is generally agreed that extent of resection predicts better PFS and our findings support this. However, this is generally true only for WHO grade I meningiomas, with a much higher rate of recurrence in WHO grade II and WHO grade III tumors, even in the setting of complete tumor resection.

Recently, the relevance of Simpson grade has come under question for even purely WHO grade I meningiomas. Sughrue et al found that even leaving a small amount of residual tumor did not affect PFS. This is not incompatible with our results, given that we combined Simpson grade 1 and 2 resections together as “subtotal resection” and higher Simpson grade resections as “subtotal” resection. It is still not completely clear whether a more aggressive surgical resection always results in higher quality of life for a given patient, especially in the aging population. This is especially true in the modern age with sophisticated stereotactic radiation modalities available for residual tumor treatment.

### Overall Survival

Not surprisingly, we found that poorer OS was associated with subtotal resection, increased MIB-1 index, and higher grade. This
TABLE 6. Biomarker Prediction of PFS/OS

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>PFS Univariate</th>
<th>PFS Multivariate</th>
<th>OS Adjusted for Age Univariate</th>
<th>OS Adjusted for Age Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1α</td>
<td>HR 1.32, P = .09</td>
<td></td>
<td>HR 1.6, P &lt; .001</td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>HR 2.52, P = .002</td>
<td>HR 2.22, P = .03</td>
<td>HR 1.3, P = .08</td>
<td></td>
</tr>
<tr>
<td>Glut-1</td>
<td>HR 1.08, P = .73</td>
<td></td>
<td>HR 1.4, P = .02</td>
<td></td>
</tr>
<tr>
<td>CA-IX</td>
<td>HR 1.31, P = .18</td>
<td></td>
<td>HR 1.1, P = .31</td>
<td></td>
</tr>
<tr>
<td>Log MiB</td>
<td>HR 3.35, P &lt; .001</td>
<td>HR 2.56, P &lt; .001</td>
<td>HR 1.9, P &lt; .001</td>
<td>HR 1.54, P = .01</td>
</tr>
<tr>
<td>Log MVD</td>
<td>HR 1.40, P = .22</td>
<td></td>
<td>HR 1.4, P = .06</td>
<td></td>
</tr>
</tbody>
</table>

*PFS, progression-free survival; OS, overall survival; MVD, microvascular density; HIF-1α, hypoxia-inducible factor-1α; Glut-1, glucose transporter-1; CA-IX, carbonic anhydrase-IX; VEGF, vascular endothelial growth factor.
parallels our PFS findings and previous literature.\textsuperscript{1,3,4,24-48} We also demonstrated shorter OS for patients with tumors expressing higher expression of HIF-1\textsubscript{a} and Glut-1. Interestingly, OS was not associated with high VEGF expression as we found for PFS, nor did high CA-IX expression or MVD influence OS. High HIF-1\textsubscript{a} expression, not high VEGF expression, was statistically significant in predicting OS. In astrocytomas, HIF-1\textsubscript{a} and VEGF levels are positively correlated with histological grade and

| TABLE 7. Patient Tumor Characteristic Prediction of PFS/OS\textsuperscript{a} |
|-----------------|-----------------|-----------------|-----------------|
| Patient Characteristic | Univariate | Multivariate | Univariate | Multivariate |
| Age | HR 1.03, \( P = .093 \) | HR 1.04, \( P < .001 \) | HR 1.04, \( P = .003 \) |
| Subtotal resection | HR 4.18, \( P = .007 \) | HR 3.55, \( P = .003 \) | \textsuperscript{b}HR 2.69, \( P = .009 \) |
| Atypical/anaplastic pathology | HR 37.5, \( P < .001 \) | HR 6.48, \( P = .031 \) | \textsuperscript{b}HR 6.88, \( P < .001 \) | HR 2.92, \( P = .023 \) |

\textsuperscript{a}PFS, progression-free survival; OS, overall survival.
\textsuperscript{b}Adjusted for age.

\textbf{FIGURE 4.} Kaplan-Meier analysis of OS of meningioma patients (\( n = 205 \)) (A) and comparing OS and high (scores 2-4) vs low (scores 0 and 1) scores for HIF-1\textsubscript{a} expression (\( P < .001 \)) (B) and Glut-1 expression (\( P = .03 \)) (C). D, Similar analysis of log of MIB-labeling index (\( P < .001 \)). OS, overall survival; HIF-1\textsubscript{a}, hypoxia-inducible factor-1\textsubscript{a}; Glut-1, glucose transporter-1.
Among patients with high-grade astrocytoma, VEGF is associated with OS when controlling for age. For meningioma, there are no studies comparing VEGF expression and OS; however, in canine meningiomas higher VEGF secretion is associated with worse OS. We also found that higher Glut-1 expression in high-grade gliomas is predictive of OS. CA-IX expression in gliomas shows a correlation with pathological grade and survival. Others have found that CA-IX was expressed in only a minority of meningiomas and failed to find a correlation with pathological grade or MIB-1-labeling index.

CONCLUSION

Similarly to previous studies, we found that meningioma patient outcome is predicted by the traditional measures of tumor grade and MIB-1 index. In addition to these factors, it appears that the biological markers HIF-1α, VEGF, and Glut-1 may predict PFS and OS. Measures of MVD may be helpful in predicting PTBE but are not predictive of tumor recurrence or patient outcome. Further studies are needed to validate these findings in an independent sample. In the future, these molecular markers may be used to better predict outcome for patients with intracranial meningiomas.

Disclosures

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**COMMENT**

Meningiomas are common intracranial tumors with an often capricious clinical course. This rather large study is an important contribution in the search for novel biological markers that could optimize the histopathological diagnosis and improve the prognostic evaluation for meningioma patients.

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