Expression of hypoxia inducible factor–1 alpha and correlation with preoperative embolization of meningiomas

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Object. Vascular endothelial growth factor (VEGF) has been implicated in meningioma tumorigenesis and growth. The production of VEGF is regulated by hypoxia inducible factor–1α (HIF-1α), especially under conditions of hypoxia. In this study, the authors examine the expression of HIF-1α and VEGF in meningiomas, with a special emphasis on conditions of hypoxia, such as preoperative embolization, and on in vitro studies in cultured cells.

Methods. Meningiomas obtained in 142 patients were studied using immunohistochemical methods to detect HIF-1α and the results were correlated with the extent or lack of preoperative embolization and expression of VEGF. Primary meningioma cell cultures were established and cell culture experiments were performed using a hypoxia chamber to stimulate HIF-1α and VEGF production. Expression of HIF-1α in primary meningioma cell cultures was measured using immunoblot assays. The VEGF secretion was measured using enzyme-linked immunosorbent assay.

Half of the meningiomas studied were positive for HIF-1α, with a strong correlation between complete embolization and HIF-1α expression. Most of the meningiomas studied expressed VEGF protein, and VEGF expression did not correlate with the degree of embolization. A strong correlation was found between VEGF and HIF-1α expression in immunohistochemical studies. Secretion of VEGF is increased by hypoxia and growth factor stimulation. In meningiomas, growth factors stimulate HIF-1α expression. The role of hypoxia is less clear.

Conclusions. The expression of HIF-1α is increased by complete preoperative embolization of meningiomas. The expression of HIF-1α also correlates with VEGF secretion in meningiomas. Growth factor and hypoxic stimulation both contribute to VEGF control, but which is most important (or whether both are equally important) will require further studies.

KEY WORDS • meningioma • hypoxia inducible factor • vascular endothelial growth factor

Materials and Methods

Patient Data and Tumor Histological Categorization

Immunohistological data are based on meningioma tissue taken...
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from 142 patients who either underwent preoperative embolization or did not require this adjuvant therapy; the patients’ data are found in Table 1. Tumors were graded as benign, atypical, or malignant according to criteria set forth in the WHO classification of brain tumors.13,23,25 with the majority being histologically benign. Various tumor locations were studied, with the most common being convexity, skull base, posterior fossa, and parasagittal. Tumors classified as nonembolized, completely embolized if there was complete obliteration of angiographically visible vessels supplying the tumor, or partially embolized if any vessels supplying the tumor were not amenable to embolization. The pial blood supply was considered intact in all tumors and was not taken into consideration in this classification.

Immunohistochemical Methods for VEGF and HIF-1α

Staining for HIF-1α. Formalin-fixed, paraffin-embedded surgical specimens were sectioned at a thickness of 6 μm, warmed to 60°C for 20 minutes, deparaffinized in xylene, and hydrated in a graded series of ethanol to H2O. Immunohistochemical studies with HIF-1α were performed using a catalyzed signal amplification system, which is based on streptavidin–biotin–horseradish peroxidase complex coupling. After deparaffinization and rehydration slides are treated with target retrieval solution at 97°C for 45 minutes according to the manufacturer’s instructions. Overnight incubation with the primary antibody H1α67 (1:1000 dilution) was performed. Nuclei were lightly counterstained with toluidine blue. Negative controls were established using nonimmune serum instead of the primary antibody. Immunoblot analysis of HIF-1α was scored as follows: 0, no staining; 1, positive staining of nuclei in less than 1% of the cell population; 2, positive staining of nuclei in 1 to 10% and/or weak cytoplasm staining; 3, positive staining of nuclei in 10 to 50% and/or with distinct cytoplasm staining; and 4, positive staining of nuclei in more than 50% of the cell population.55

Staining for VEGF. For VEGF immunohistochemical analysis, sections were deparaffinized with xylene and dehydrated in a graded series of alcohols to H2O. Antigen retrieval was performed by microwave heating of sections immersed in 0.01 mol/L citrate buffer (pH 6). Endogenous peroxidase was blocked by incubation in 0.3% H2O2. Nonspecific binding was blocked with 10% normal goat serum. The specimens were then incubated overnight with anti-VEGF Ab-1 (1:50 dilution). Secondary antibodies were added and ABC incubations were performed with the ABC kit. The final reaction was completed with treatment of the sections in the peroxidase substrate solution DAB. After counterstaining with toluidine blue, sections were dehydrated in graded series of alcohol and xylene, and coverslips were applied with Dupex. Negative controls were obtained by replacing the primary antibody with nonimmune serum, with all other steps being performed as described earlier. The immunohistochemical analysis of VEGF was scored as follows: 0, no staining; 1, less than 1% positively stained cells; 2, 1 to 10% positively stained cells; 3, 10 to 50% positively stained cells; and 4, greater than 50% positively stained cells.

Measurement of Proliferation Index

The proliferation index was calculated after the immunohistochemical staining of meningioma cells with the MIB-1/Ki67 mAb by using methods described earlier. A total of 1000 cells in four randomly selected quadrants of high-power fields were counted and the ratio of positive cells/total number of cells counted was calculated as the proliferation index.

Primary Meningioma Cell Culture

Tumor specimens were obtained at the time of operation in patients with meningiomas. Establishment of primary meningioma cultures has been previously described in detail.15,16 Briefly, meningiomas were taken immediately from the operating room, digested with collagenase, and placed in modified Eagle medium containing 10% fetal bovine serum. Only passages 1 and 2 were used in experiments for this study. Confirmation of the cultured cells as meningioma cells was made using electron microscopy and light microscopy.15,16

Hypoxia Chamber Cell Growth Studies

For hypoxia experiments, cells were plated on 100-mm tissue culture dishes until they reached 80% confluence. The culture medium was changed immediately before placing the dishes in an anaerobic culture chamber containing an H2 and Pd catalyst to remove all traces of O2; cells were treated for 4 to 24 hours. On breaking the seal of the hypoxia chamber, 1 ml of medium from each group was removed and placed on ice. This was stored at −20°C until protein measurement and ELISA for VEGF were performed. The remaining medium was decanted and the plates were washed with ice-cold Dulbecco PBS and placed in an ice-water bath to minimize the proteasomal degradation of HIF-1α. Cells were scraped from the culture dishes and cell protein was isolated for Western blot analysis as described later.

Immunooassay for VEGF

Medium containing cell supernatant was harvested from experimental groups. The VEGF concentrations in the growth medium were measured using a commercially available ELISA. This system uses ELISA plates precoated with an anti-VEGF murine mAb. Samples to be tested, as well as standards for VEGF, were added to the plate wells in triplicate. After washing away the unbound substances, an enzyme-linked PAB specific for VEGF was added to the wells. The plates were again washed, substrate solution was added, and the color was allowed to develop. Absorbance at 450 nm was measured and corrected using the 540-nm reading on a microplate reader. Data analysis was performed using commercially available software. A standard curve was generated by plotting absorbance compared with VEGF standard concentration by using linear and nonlinear regression. The standard curve was linear from 0.015 to 2 ng/mL. The concentration of VEGF in the samples is calculated by interpolation from the standard curve. Total protein is determined to normalize ELISA for cell growth.

Protein Isolation and Western Blot Analysis

Isolation of protein from primary meningioma cultures for HIF-1α determination was performed on cells treated in the hypoxia chamber as described earlier. After the growth medium was decanted from these cells, the monolayer was washed twice with ice-cold Dulbecco PBS. The plates were kept in an ice-water bath while the cells were dislodged into the residual Dulbecco PBS by using a cell scraper; cells were then transferred to a microcentrifuge tube. The cells were pelleted at 5000 g for 5 minutes at 4°C, the residual Dulbecco PBS was aspirated, and 150 μl of ice-cold buffer (20 mM HEPES [pH 7.6], 25 mM KCl, 0.5% vol/vol IPEGAL) was added to the pellet. The cells were suspended in lysis buffer by repeatedly passing the solution through a chilled tuberculin syringe. The suspension was incubated on ice for 20 minutes to ensure complete lysis. Cell debris was pelleted by centrifugation at 21,100 G at 4°C for 10 minutes. The supernatant containing the crude cell extract was removed and stored at −20°C until use.

The total protein concentration of the cellular extract described earlier was determined spectrophotometrically in 96-well plates by using the DC total protein assay kit and read using a microplate reader. Equal amounts of protein (approximately 50 μg) were resolved using an SDS-PAGE slab gel (4% stacking, 7.5% resolving) and transferred to a nitrocellulose membrane. The membrane was blocked and probed in Blotto (50 mM Tris [pH 7.5], 0.9% NaCl, 5% nonfat dry milk, and 0.1% Tween 20) by using a mouse IgG anti-human HIF-1α mAb (H72320, 1:1600 dilution) and a mouse IgG antiaxin mAb (1501, 1:5000 dilution). The Western blots were visualized using enhanced chemiluminescence reagents and x-ray film.

Statistical Analysis

Chi-square analysis and Spearman rank-order correlation tests were performed to determine if a relationship existed among HIF-1α expression, embolization, and VEGF expression. Comparisons
of hypoxic and normoxic values for VEGF secretion were made using the Student t-test, with significance set at probability levels less than 0.05.

Sources of Supplies and Equipment

The catalyzed signal amplification system and the target retrieval solution were purchased from Dako Corp., Carpinteria, CA. The primary antibody H1α67 was obtained from Novus Biologicals, Inc., Littleton, CO. The anti-VEGF Ab-1 pAb was supplied by Calbiochem, Cambridge, MA. The Vectastatin ABC kit and the DAB substrate were acquired from Vector Laboratories, Burlingame, CA. The MIB-1/Ki67 mAb was obtained from Santa Cruz Biotechnology, Santa Cruz, CA. The tissue culture dishes were purchased from Becton-Dickinson, Franklin Lakes, NJ, as were the GasPak Plus anaerobic culture chamber and the GasPak Plus H2- CO2 generators. The Dupex coverslip adhesive was acquired from Bio-Rad Laboratories, Hercules, CA, as was the DC total protein assay kit. The nitrocellulose Immunoblot-P membrane was purchased from Millipore, Bedford, MA. The mouse IgG anti–human HIF-1α mAb was obtained from Transduction Laboratories, Lexington, KY. The mouse IgG antiantiactin mAb was acquired from Chemicon International, Temecula, CA. The chemiluminescent reagents were supplied by Amersham Pharmacia, Piscataway, NJ. The X-OMAT film was purchased from Eastman Kodak, Rochester, NY.

Results

Patient Demographics and Tumor Characteristics

Of the 142 patients studied, 67% were women and the age range was from 22 to 88 years with a median age of 56 years. Ninety patients (63%) did not require preoperative embolization. Of the 52 patients who underwent preoperative embolization, in 32 there was complete obliteration of angiographically visible vessels supplying the tumor. In the remaining patients the embolization was considered incomplete because some vessels were not amenable to embolization.

There was no statistical difference between sexes, ages, and selection for tumor embolization, either complete or incomplete. The majority of tumors were histologically benign, with only nine atypical and no malignant tumors studied. There was no correlation between selection for embolization and eventual tumor histological findings. Various tumor locations were studied, with the most common being convexity, skull base, posterior fossa, and parasagittal. There does not seem to be a convincing pattern of tumor location determining which tumors were selected for preoperative embolization. Similarly, location did not seem to be correlated with partial or complete embolization.

Expression of HIF-1α and VEGF in Embolized Meningiomas

According to the scoring system described earlier, there was a wide variation in expression of HIF-1α in meningiomas obtained in human patients, although expression was evenly distributed across groups. With scores of 0 and 1 designated as negative for HIF-1α, and scores of 2, 3, and 4 as positive, 53% of tumors were immunohisto logically positive and 47% were negative for HIF-1α. We found that VEGF is expressed in 83% of meningiomas according to a similar grading scale (Table 2 and Fig. 1).

The effect of embolization on HIF-1α and VEGF expression is shown in Table 3. Half of the meningiomas obtained from patients who did not undergo preoperative embolization were positive for HIF-1α. The same was true when looking at the embolization groups as a whole. When a comparison was made between complete and partial embolization, however, a difference was found: 23 (72%) of 32 meningiomas were positive for HIF-1α in the completely embolized tumors. This was statistically different from tumors that were not embolized, according to chi-square testing (p = 0.053) and it was positively correlated according to the Spearman rank-sum correlation (p = 0.362, p = 0.0003).

Curiously, only five (25%) of 20 partially embolized tumors demonstrated HIF-1α expression on immunohistochemical studies. Once again, this was statistically different from the nonembolized groups, according to chi-square testing (p = 0.027) and it was positively correlated according to Spearman rank-sum correlation (p = 0.252, p = 0.117). Histological examination of all lesions demonstrated no frank, discrete areas of tumor necrosis on any section.

TABLE 2
Expression of HIF-1α and VEGF in 142 meningiomas

<table>
<thead>
<tr>
<th>Extent of Staining*</th>
<th>HIF-1α†</th>
<th>VEGF‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>HIF-1α†</td>
<td>34 (24)</td>
<td>33 (23)</td>
</tr>
<tr>
<td>VEGF‡</td>
<td>8 (6)</td>
<td>17 (12)</td>
</tr>
</tbody>
</table>

* Values are given as the number of tumors in each staining category, with percentages in parentheses. 0 = negative for HIF-1α or VEGF; 2–4 = positive for HIF-1α or VEGF. See Immunohistochemical Methods for VEGF and HIF-1α for a complete explanation of staining grades.
† Total for 0 and 1, 47%; total for 2 through 4, 53%.
‡ Total for 0 and 1, 17%; total for 2 through 4, 83%.
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Fig. 1. Photomicrographs showing immunohistochemical findings for VEGF and HIF-1α in tumor samples. a: Human meningioma stained for VEGF, Grade 4 on a scale of 0 to 4, with 4 being the highest level of expression of this protein. We used DAB as the developing substrate and toluidine blue as the counterstain. Original magnification × 10. b: Negative control, same meningioma, original magnification × 10. c: Human meningioma section stained for HIF-1α, Grade 4 on a scale of 0 to 4, with 4 being the highest level of expression of this protein. We used DAB as a substrate, counterstained with toluidine blue, original magnification × 20. d: Negative control, same tumor, original magnification × 10. e: Different meningioma, Grade 4, with more than 50% of cells with nuclear staining. Similar immunohistochemical method demonstrating nuclear staining for HIF-1α. Original magnification × 40. f: Negative control without nuclear staining, original magnification × 40. g: An MIB-1–labeled atypical meningioma with a proliferation index of approximately 15%.
including the partially or completely embolized tumors. Very small areas of microinfarction were occasionally present in various tumors without a clear pattern of differences of HIF-1α expression correlating with these areas.

A similar analysis of VEGF expression in relation to embolization demonstrated roughly 80% expression of VEGF in all groups regardless of the extent or lack of preoperative embolization; there was no statistically significant difference between groups. Because VEGF expression is regulated by HIF-1α under many physiological conditions, the correlation of expression of HIF-1α, VEGF, and the VEGF receptor was examined. Correlation of HIF-1α and VEGF grading scales revealed a strong correlation between scores according to chi-square testing (p < 0.0004, Table 4). An analysis of VEGF receptor expression in relation to HIF-1α expression demonstrated no significant correlation of expression (data not shown).

Proliferation Index and Embolization

To assess the effect of embolization on the proliferation index, MIB-1 labeling was performed in 82 benign tumors (35 unembolized, 20 partially embolized, and 27 completely embolized) and all nine atypical meningiomas (five unembolized, two partially embolized, and two completely embolized). This assures that there is no intrinsic difference in tumor biology between the tumors that were embolized, either completely or partially, and those that were not. Not surprisingly, the atypical meningiomas demonstrated a higher proliferation index than the benign ones (Fig. 1). There was no statistical difference, however, between the proliferation index and embolization status (Table 5).

Production of VEGF in Meningioma Cells

To correlate VEGF production to HIF-1α expression in meningioma cells, measurements of VEGF were made using the culture medium of the same cells used for the HIF-1α expression experiments described earlier. Five primary meningioma cell lines (M1–M5) were used for these experiments. Tumors were subjected to hypoxic conditions and compared with similar cells grown in normoxic conditions as described earlier. Medium taken from each individual culture dish containing a given cell line was examined using the ELISA for VEGF protein.

Production of VEGF was normalized to total protein in the medium. The baseline VEGF was relatively low, especially when compared with glioma cell lines (data not shown). The VEGF production was significantly increased under hypoxic conditions for all tumors (Student t-test, p < 0.05), but once again much less than that demonstrated in the glioma cell lines (Fig. 2).

The relationship between increasing doses of EGF or PDGF and a 4-hour exposure to conditions of normoxia or hypoxia was studied next. The primary meningioma cell line M5 was used, because it demonstrated the highest level of VEGF secretion (Fig. 2). The addition of EGF and PDGF increases VEGF secretion in a dose-dependent manner by using concentrations of 5, 10, and 20 ng/ml of growth factor. Secretion of VEGF increases in hypoxic conditions as it does after stimulation by both EGF and PDGF (p > 0.05), as indicated by results of one-way analysis of variance and Fisher protected least significant difference tests in control groups (Fig. 3).

Expression of HIF-1α in Meningiomas

The supernatant from the same five primary meningioma cell lines mentioned earlier was analyzed using Western blots for HIF-1α expression and compared with U251 glioma cell lines subjected to similar conditions (Fig. 4). No expression was seen in the case of the primary meningiomas (only the hypoxic cells are shown here). The U251 cells show baseline expression of HIF-1α, which is significantly increased after 4 hours of hypoxic conditions; we used CoCl2-activated HeLa cells as a control. Because CoCl2 potentiates HIF-1α expression in a number of cell lines,1 it was used to confirm that this system was func-

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**TABLE 3**

<table>
<thead>
<tr>
<th>Extent of Embolization</th>
<th>No. of Tumors</th>
<th>HIF-1α (%)</th>
<th>VEGF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>total</td>
<td>142</td>
<td>67 (47)</td>
<td>75 (53)</td>
</tr>
<tr>
<td>none</td>
<td>90</td>
<td>43 (48)</td>
<td>47 (52)</td>
</tr>
<tr>
<td>embolized</td>
<td>52</td>
<td>24 (46)</td>
<td>28 (54)</td>
</tr>
<tr>
<td>completely</td>
<td>32</td>
<td>9 (28)</td>
<td>23 (72)*</td>
</tr>
<tr>
<td>partially</td>
<td>20</td>
<td>15 (75)†</td>
<td>5 (25)</td>
</tr>
</tbody>
</table>

* According to chi-square testing (p = 0.053); positively correlated by Spearman rank-sum correlation (p = 0.362, p = 0.0003) comparing completely embolized and nonembolized groups.

† According to chi-square testing (p = 0.027); positively correlated by Spearman rank-sum correlation (p = 0.252, p = 0.117) comparing partially embolized and nonembolized groups.

**TABLE 4**

<table>
<thead>
<tr>
<th>VEGF Staining</th>
<th>HIF-1α Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>142</td>
<td>34 (24) 33 (23)</td>
</tr>
<tr>
<td>8 (6)</td>
<td>0 0 3 2</td>
</tr>
<tr>
<td>17 (12)</td>
<td>4 6 5 1 1</td>
</tr>
<tr>
<td>41 (29)</td>
<td>16 12 6 6 1</td>
</tr>
<tr>
<td>41 (29)</td>
<td>5 8 10 5 13</td>
</tr>
<tr>
<td>4 (25)</td>
<td>6 7 1 6 15</td>
</tr>
</tbody>
</table>

* Values represent numbers of meningiomas (%). p < 0.0004 according to chi-square testing.

**TABLE 5**

<table>
<thead>
<tr>
<th>Meningioma proliferation index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Type</td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>benign</td>
</tr>
<tr>
<td>mean ± SD</td>
</tr>
<tr>
<td>no. of lesions</td>
</tr>
<tr>
<td>atypical</td>
</tr>
<tr>
<td>no. of lesions</td>
</tr>
</tbody>
</table>

* SD = standard deviation.
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Expression of HIF-1α in the M5 primary meningioma cell line was studied after exposure to PDGF-BB (10 ng/ml) over time under normoxic conditions (Fig. 5). These cells show PDGF-BB–induced expression of HIF-1α beginning at 2 hours, with maximal expression between 3 and 5 hours. The control group was exposed to conditions similar to those for the 6-hour group, but without PDGF.

Discussion

Meningiomas and VEGF

We have demonstrated that a high percentage of meningiomas were immunohistochemically positive for VEGF protein. This is not surprising, because many other investigators have found similar results.2,11,31,34 The concentrations of VEGF measured in our tumor cells grown in normoxic conditions were similar to those found in other studies46 and measured using similar methods. Takano, et al., and others11,22 have noticed similar differences between glioblastoma multiforme (very high expression) and meningioma (much lower expression) to those shown in our paper. The question of how clinically relevant this finding is has been the subject of conflicting opinion and differing experimental findings. A correlation between meningioma grade and VEGF expression has been demonstrated.25,26 We found no such correlation; however, this could be explained by the small numbers of malignant and atypical meningiomas in our study. Nevertheless, others2,7,11,31,34 also have found no difference in VEGF expression and meningioma degree of malignancy, verifying our results.

Even more confounding, in studies that have shown a correlation between meningioma grade and VEGF expression, it did not always correlate with increased microvascular density, vascularity, or invasiveness.22 On the other hand, other investigators39 have shown a high correlation between VEGF expression and neovascularization in meningiomas. A correlation between VEGF content and meningioma malignancy grade but the lack of a correlation with microvessel density indicates that VEGF could serve functions other than those pertaining to angiogenesis in meningiomas, such as autocrine stimulation of the tumor cells.22 This is questionable, because we have shown that VEGF does not stimulate meningioma growth in culture (unpublished data).

The elevated expression of VEGF linked to peritumoral edema has been demonstrated;10,18,35 similar observations of VEGF expression and the recurrence rate of meningiomas have been made.30 It has been suggested that VEGF secreted from tumor cells might promote the proliferation of microvessels and feeding pial arteries, as well as increase edema (with resultant adhesion to the surrounding brain tissue), which would in turn increase recurrence of the tumor. This is supported by evidence showing a link between VEGF expression, arterial tumor supply, and peritumoral brain edema.3 Others51 have suggested that VEGF expression contributes to peritumoral brain edema in meningiomas only when a cerebral–pial blood supply exists.51

Control of VEGF expression in meningiomas is not well understood; VEGF expression can be increased by growth factors such as EGF and PDGF, or decreased by dexamethasone in meningiomas in culture.47 We have
made similar observations in this paper. It has been reported that HIF-1α induces the transcription of VEGF in most cell types studied. Expression of HIF-1α has been demonstrated in malignant gliomas, but no studies of meningiomas have been performed.

**Hypoxia Inducible Factor–1α**

Hypoxia-inducible factor–1 is composed of two heterodimeric subunits, HIF–1α, also known as aryl hydrocarbon receptor nuclear translocator, and HIF–1β. These subunits are members of the basic-helix-loop-helix Per Arn family of proteins. The NH₂-terminal basic-helix-loop-helix domain is responsible for heterodimerization and DNA binding. Together they regulate VEGF transcription or, more accurately, HIF–1 binds the hypoxia response element and induces the transcription of a number of well-characterized genes that help cells cope with low O₂ conditions, including VEGF and almost every gene in the glycolytic pathway.

At the messenger RNA level, HIF–1α and HIF–1β are constitutively expressed and do not seem to be significantly modified by hypoxia. Nevertheless, although HIF–1β protein is found in normoxic cells, almost no HIF–1α can be detected under these conditions. In normoxic conditions (20% O₂), HIF–1α is rapidly degraded by proteasomal activity through a mechanism requiring targeting by the VHL protein for polyubiquitination. Recent studies have shown that the interaction of HIF–1α with the VHL protein is regulated through hydroxylation of a proline residue (HIF–1α P564) by the enzyme prolyl hydroxylase. This reaction requires dioxygen and Fe as cosubstrates, suggesting that this system acts as a cellular O₂ sensor.

The VHL protein forms a complex with elongins B and C, Rbx1, and cullin-2 through the elongin C–binding domain. This acts as the recognition component of an E3 ubiquitin ligase complex for polyubiquitination. The polyubiquitinated HIF–1α protein is degraded by the ubiquitin-dependent 26S proteasome through binding to the prosome macropan subunit alpha type 7 (PSMA7) subunit of the proteasome. During conditions of low O₂ tension (1–2% O₂), this degradation is inhibited by a mostly unknown mechanism, leading to increased HIF–1α which translocates to the nucleus to activate gene transcription.

Various human cancers have increased levels of HIF–1α compared with their respective normal tissue of origin. Hypoxia-inducible factor–1 is thought to be involved in tumorigenesis through a number of different mechanisms. On one hand, hypoxia induces the activation of HIF–1, which increases the expression of several glycolytic enzymes and hence the glycolysis rate. This allows the cells to survive through an adaptive modification of their energy metabolism. On the other hand, HIF–1 is involved in the establishment of vascular supply, and neoangiogenesis can be triggered by tumor cells becoming hypoxic while enlarging. In the case of meningiomas, it is possible that the tumor grows slowly enough that it never outgrows its blood supply. In this scenario, HIF–1 would not be necessary for neovascularization. Only half of the meningiomas in this study were immunohistologically positive for HIF–1α, and only a few were expressing HIF–1α protein at the time of initial resection, according to immunoblotting techniques (data not shown). It follows that if the tumor became hypoxic, as in the case of embolization, HIF–1α expression would be necessary.

**Hypoxia, Embolization, and Meningiomas**

Preoperative embolization of meningiomas results in intratumoral necrosis. The characteristic features of embolization-induced necrosis include a sharp outline of confluent areas of necrosis, the lack of an overall background of cellular anaplasia, and the frequent coexistence of features of cellular ischemia. We believed that this was a situation that would stimulate HIF–1α expression. Interestingly, only complete embolization resulted in increased HIF–1α expression, leading us to speculate that partial embolization leaves enough of a blood supply to prevent tumor hypoxia.

Necrosis is known to be an important histological hallmark of atypicality and malignancy in meningiomas. Unfortunately, in our group there were relatively small numbers of these higher-grade tumors, and no conclusions related to necrosis or HIF–1α and VEGF expression can be made. This should be investigated in high-grade meningiomas, however, because the highest levels of VEGF expression in high-grade gliomas are found immediately adjacent to necrotic foci in pseudopallisading cells.

The possibility that embolization, apart from necrosis,
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will induce increased proliferative activity in meningiomas was first raised by Paulus, et al.\(^3\) In their study of 15 embolized meningiomas, four tumors exhibited increased Ki-67 labeling. This was confirmed by Ng, et al.,\(^3\) who found increased MIB-1 and proliferating cell nuclear antigen labeling indices in tumors with necrosis; however, in general there was no obvious increase in mitotic activity. These authors hypothesized that this represented a temporary reactive response to necrosis, because none of these tumors ultimately carried a worse prognosis for the patient. These effects could potentially be mediated by HIF-1\(\alpha\) because HIF-1 induces the transcription of VEGF and almost every gene in the glycolytic pathway.\(^8,37,49\) We could see no difference in the MIB-1 labeling index between tumors that were either unembolized or partially or completely embolized preoperatively. We found no correlation between HIF-1\(\alpha\) or VEGF expression and the MIB-1 labeling index.

Malignant as well as benign meningiomas can become unresectable when they recur and invade neighboring structures such as cranial nerves. Under such circumstances, radiation, chemotherapy, and hormone therapy have also proven to be of little benefit to these patients.\(^4\) Therefore, an understanding of potential pathways, such as VEGF/HIF-1, that are potentially involved in malignant transformation, invasion, and peritumoral edema, could result in clinically relevant strategies for treatment of these tumors.

**Conclusions**

Half of all the meningiomas studied were positive for HIF-1\(\alpha\), with a strong correlation between complete embolization and HIF-1\(\alpha\) expression. There was also a strong correlation between VEGF and HIF-1\(\alpha\) expression, but no correlation between VEGF levels and tumor embolization. From these studies it is not entirely clear if HIF-1\(\alpha\) is required for VEGF secretion in meningiomas. Growth factor and hypoxic stimulation both contribute to VEGF control, but which is most important (or whether both are equally important) will require further studies.

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**References**

44. Sutter CL, Laughner E, Semenza GL: Hypoxia-inducible factor 1α protein expression is controlled by oxygen-regulated ubiquitination that is disrupted by deletions and missense mutations. Proc Natl Acad Sci USA 97:4748–4753, 2000
46. Tsai JC, Hsiao YY, Teng LJ, et al: Regulation of vascular

endothelial growth factor secretion in human meningioma cells. 


Proc Nat Acad Sci USA 92:5510–5514, 1995

Cancer 89:1102–1110, 2000

Cancer 85:936–944, 1999


Cancer 88:2606–2618, 2000

Cancer Res 59:5830–5835, 1999

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