Are there attacking points in the eicosanoid cascade for chemotherapeutic options in benign meningiomas?

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Object. The current treatment for recurrent or malignant meningiomas with adjuvant therapies has not been satisfactory, and there is an intense interest in evaluating new molecular markers to act as therapeutic targets. Enzymes of the arachidonic acid (AA) cascade such as cyclooxygenase (COX)-2 or 5-lipoxygenase (5-LO) are upregulated in a number of epithelial tumors, but to date there are hardly any data about the expression of these markers in meningiomas. To find possible targets for chemotherapeutic intervention, the authors evaluated the expression of AA derivatives at different molecular levels in meningiomas.

Methods. One hundred and twenty-four meningioma surgical specimens and normal human cortical tissue samples were immunohistochemically and cytochemically stained for COX-2, COX-1, 5-LO, and prostaglandin E receptor 4 (PTGER4). In addition, Western blot and polymerase chain reaction (PCR) analyses were performed to detect the presence of eicosanoids in vivo and in vitro.

Results. Sixty (63%) of 95 benign meningiomas, 21 (88%) of 24 atypical meningiomas, all five malignant meningiomas, and all normal human cortex samples displayed high COX-2 immunoreactivity. All cultured specimens and IOMM-Lee cells stained positive for COX-2, COX-1, 5-LO, and PTGER4. The PCR analysis demonstrated no changes in eicosanoid expression among meningiomas of different World Health Organization grades and in normal human cortical and dura mater tissue.

Conclusions. Eicosanoid derivatives COX-1, COX-2, 5-LO, and PTGER4 enzymes show a high universal expression in meningiomas but are not upregulated in normal human cortex and dura tissue. This finding of the ubiquitous presence of these enzymes in meningiomas offers an excellent baseline for testing upcoming chemotherapeutic treatments. (DOI: 10.3171/FOC-07/10/E8)

KEY WORDS • chemotherapy • eicosanoid • meningioma •
polymerase chain reaction • Western blot

MENINGIOMAS are usually benign tumors that arise from the leptomeningeal cells of the arachnoid membrane surrounding the brain and spinal cord. However, recurrence after seemingly complete surgical removal occurs in eight to 15% of cases, and tumor control rates achieved with radiotherapy are only 80%.^{20,35,36,53} Moreover, a surgical cure is not always achievable in meningiomas involving the skull base or vital neurovascular structures. Results achieved with chemotherapeutic treatments in the past were not convincing, and even drugs such as temozolomide—which have shown high efficacy

against malignant brain tumors—have failed to inhibit the growth of refractory meningiomas in Phase II studies.^{7,26,39,45} The development of novel treatment strategies based on molecular information has not yet been successfully translated into common clinical practice.

Arachidonic acid is a v6 polyunsaturated fatty acid that is converted into biologically active lipid compounds called eicosanoids. Eicosanoids constitute a large family of biologically active lipid mediators produced by two enzyme classes: the cyclooxygenases (COX-1 and COX-2) and the lipoxygenases (5-LO, 12-LO, and 15-LO). Both classes catalyze the same enzymatic reaction that occurs in the synthesis of PG₂ and PGH₂, which are successively metabolized to PGE₂, PGD₂, PGF₂, thromboxane A₂, and prostacyclin PGI₂ (Fig. 1).¹²

The crucial role of COX enzymes has been clearly demonstrated in human colon cancer cells, in which COX-2 is often overexpressed.^{30,40} Cyclooxygenase inhibitors,

Abbreviations used in this paper: AA = arachidonic acid; COX = cyclooxygenase; LO = lipoxygenase; PBS = phosphate-buffered saline; PCR = polymerase chain reaction; PG = prostaglandin; PTGER4 = prostaglandin E receptor 4; WHO = World Health Organization.

FIG. 1. Simplified diagram demonstrating the AA cascade and the enzymes produced. EGFR = epidermal growth factor receptor; IL = interleukin; NFκB = nuclear factor-κB; NSAIDs = nonsteroidal antiinflammatory drugs; PLA2 = phospholipase A2.

such as nonsteroidal antiinflammatory drugs and COX-2-specific inhibitors, can reduce the incidence of colorectal cancer in humans and experimental animal models, and can decrease the number and size of polyps in patients with familial adenomatous polyposis.¹³ Recently, gliomas and meningiomas were also found to overexpress both COX and LO enzymes compared with normal brain tissue.^{6,8,37} Matsuo and coworkers³² reported that the staining intensity in glioblastomas was relatively weak, but that meningiomas and WHO Grade II/III astrocytomas were strongly positive for COX-2. Although much attention has been focused on the role of COX-derived metabolites in cancer development and progression, accumulating evidence suggests that 5-LO-derived eicosanoids may play an equally important role. 5-lipoxygenase mRNA and/or protein was found to be overexpressed in human breast, pancreatic, or colon cancer cells.^{17,21,49} Boado et al.³ reported increased expression of 5-LO mRNA in three of three meningioma surgical specimens examined.

Most tumors that express COX have been found to contain high levels of PGE₂.¹⁰ Presumably, this especially bioactive lipid product of COX is responsible for some of the proneoplastic effects mediated by the enzymes such as inducing growth, migration, and invasiveness in colorectal carcinoma cells.⁵ Little is known of the production of PGs in intracranial tumors; some authors have reported elevated PGE₂ levels in brain tumors in general and significantly higher PGE₂ level in meningiomas.^{9,25} In contrast, other authors have reported no increase brain tumors' production of PGE₂.¹⁴ In the present study, we demonstrate the ubiquitous expression of AA derivatives in human meningiomas with immunohistochemical staining, Western blot analysis, and PCR.

Materials and Methods

Tumor Specimens and Cell Culture

Meningioma surgical specimens were obtained from the Neurosurgical Department in accordance with regulations of the Ethics Committee of the University of Tübingen. Primary cultures were obtained from tumor tissue samples within 15 minutes of surgical removal. The samples were first washed in PBS, reduced and mashed through a filter and placed in Dulbecco modified Eagle's medium with fetal bovine serum, 2 mmol/L L-glutamine, and 0.1% 10 mg/ml gentamicin (Invitrogen). The cells were plated in 12.5-mm² tissue culture flasks and incubated at 37°C in a humidified atmosphere of 5% CO₂. The medium was changed every 3 to 4 days, and cultures were split using 300-μl accutase (PAA). Viable cells were frozen in liquid nitrogen in 90% medium/10% dimethyl sulfoxide. As there is no PGE₂ antibody commercially available, the subtype 4 of the prostaglandin receptor E2 was selected for the studies because it is the only prostaglandin E2 receptor subtype that is expressed in brain. The IOMM-Lee cell line was a kind gift from Dr. Anita Lal (Department of Neurological Surgery, University of California, San Francisco).

Immunohistochemical Staining

Four-micron sections containing human meningiomas were cut from formalin-fixed tissue embedded in paraffin blocks. Normal cerebral cortex (Biochain Inc.), colon, and tonsil tissues were used as positive controls. Slides containing tissue were deparaffinized by bathing them in a series of histolene (Engelbrecht) and alcohol solutions. Vectastain Elite Universal Kits (Vector Laboratories) were used according to the manufacturer's protocol. Briefly, the

slides were treated with an antigen unmasking solution (citrate buffer solution at pH 6.0), rinsed with PBS, and bathed in a 3% H₂O₂ solution for 10 minutes, followed by blocking with avidinbiotin (Vector Laboratories). Slides were incubated overnight at 4°C with primary COX-2 mouse monoclonal antibody (160112, Cayman Chemical) at a dilution of 1:50, COX-1 mouse monoclonal antibody (160110, Cayman Chemical) at a dilution of 1:100, 5-LO rabbit polyclonal antibody (101775, Cayman Chemical) at a dilution of 1:100, and PTGER4 rabbit polyclonal antibody (160402, Cayman Chemical) at a dilution of 1:50. After the samples were incubated with the primary antibody and washed twice with PBS, biotinylated secondary antibody was applied, and the samples were incubated with premixed avidin–biotin peroxide complex (Vector Laboratories). The final immunohistochemical staining was performed using Vector NovaRED (Vector Laboratories) to obtain a reaction to the avidin–biotin complex. Sections were counterstained with hematoxylin and examined under light microscopy.

The immunohistochemical samples were graded using a five-point scoring system to describe the percentage of cells that demonstrated positive staining, as previously published.⁴⁴ The scores were as follows: 0, no staining noted; 1, less than 1% of cells stained; 2, 1 to 10% of cells stained; 3, 11 to 50% of cells stained; and 4, more than 50% of cells stained.

Immunocytochemical Staining

Immunocytochemical analysis was performed on meningioma cells growing in a monolayer fashion in culture. The cells were subjected to treatment, plated in four-well glass slides, and allowed to remain in growth media for 2 to 3 days as described in the previous section. After removal of the growth media, the slides were rinsed with PBS for 5 minutes. Methanol maintained at –20°C was added to each well for 10 minutes. After the methanol had been removed, the slides were rinsed twice with PBS and bathed in 3% H₂O₂ solution for 5 minutes to quench endogenous peroxidases. The incubation and staining protocols are identical to the immunohistochemical stainings described earlier.

Protein and RNA Isolation

Cytoplasmic and nuclear protein and RNA were isolated from surgical specimens and primary cell cultures using the protein and RNA isolation system (PARIS, Ambion, Inc.) according to the manufacturer's protocol. The nuclear and cytoplasmic protein and RNA was measured spectrophotometrically. The probes were stored at –80°C until use.

Reverse Transcription and PCR

Ribonucleic acid samples were treated with DNase using the DNasefree kit (Ambion, Inc.) prior to reverse transcription–PCR analysis. A 0.4-μg sample of cytoplasmic and nuclear RNA was reverse-transcribed using random hexamer primers and the Transcriptor First Strand cDNA Synthesis kit (Roche) according to the manufacturer's protocol. The PCR was performed with 50 ng of cDNA in a 50-μl reaction mixture containing FastStart Taq DNA Polymerase dNTPack (Roche) according to the manufacturer's protocol, with COX-2, COX-1, ACTB (loading

control), PTGER4 primer (SuperArray), and 5-LO primer: forward, 5'-TCTCAAGCAACACCGACGTAAAG-3'; reverse, 5'-TGCATGAAGCGGTTGATGAA-3' designed based on the sequence of human 5-LO cDNA.⁴⁰ The PCR conditions for COX-2, COX-1, ACTB, and PTGER4 were 95°C for 15 minutes, 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, and one cycle of 72°C for 10 minutes. For 5-LO the conditions were 40 cycles at 95°C for 45 seconds, 60°C for 1 minute, 72°C for 1 minute, and one cycle at 72°C for 10 minutes. Polymerase chain reaction products (16 μl) were subjected to electrophoresis on 10% TBE-Gel, and stained with the SilverQuest Silver Staining Kit (LC6070, Invitrogen, Inc.). The intensity of expression for each reaction was analyzed using a semiquantitative grading system as follows: Grade 0, no expression; Grade 1, minimal expression; Grade 2, moderate expression; Grade 3, marked expression; and Grade 4, strong expression.

Western Blot Analysis

Equal amounts of protein (~ 100 μg) were resolved using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Nupage Bis-Tris-Gel 4–12%, Invitrogen, Inc.) and transferred to polyvinylidene difluoride Hybond-P membrane (Invitrogen, Inc.). The membrane was blocked and probed using a COX-2 mouse monoclonal antibody (160112, Cayman Chemical) at a dilution of 1:1000, COX-1 mouse monoclonal antibody (160110, Cayman Chemical) at a dilution of 1:100, 5-LO rabbit polyclonal antibody (101775, Cayman Chemical) at a dilution of 1:250, and PTGER4 rabbit polyclonal antibody (160402, Cayman Chemical) at a dilution of 1:200. An antibody to β-actin (1:1000; Ab8226, Abcam) was used to confirm equal loading among the samples. The Western blots were visualized using the Western Breeze Chromogenic Kit (Invitrogen, Inc.). The intensity of expression for each reaction was analyzed using the same semiquantitative grading system already described.

Statistical Analysis

Results are expressed as the means ± the standard error of the means. Statistical analysis was performed using the unpaired t-test. Probability values less than 0.05 were considered statistically significant.

Results

Patient Demographics and Tumor Characteristics

Tumor samples were obtained in 124 patients. The patients ranged from 22 to 88 years of age (mean 61 years), and 98 (79%) were women. Although 95 tumors (77%) were histologically benign, there were 24 atypical (19%) and five anaplastic meningiomas (4%). For the in vitro immunohistochemical staining studies, seven primary cell cultures were grown in monolayer cultures. Seventy-one percent of the cell lines had been obtained in female patients; the cell lines were derived from two benign, one atypical, one chordoid meningioma, and two malignant (IOMM-Lee) meningiomas. The primary cell cultures were immunohistochemically stained with epithelial membrane antigen and vimentin antibody to verify that they were

meningioma cells. A strong correlation between the Ki 67 proliferation index and the growth of tumor cells in culture has been seen ($p < 0.001$; Fig. 2).

Immunohistochemical Analysis of Eicosanoids in Meningiomas

Immunohistochemical staining for COX-2 was performed in 124 human meningioma surgical specimens, with tonsil tissue used as a positive control. Ninety-five benign (77%), 24 atypical (19%), and five (4%) anaplastic meningiomas stained positively for COX-2 (Table 1). The COX-2 immunoreactivity was found exclusively in the cytoplasm of all meningiomas studied. In fact 60 (63%) of the 95 benign meningiomas, and 21 (88%) of the 24 atypical meningiomas exhibited Grade 4 staining (Fig. 3D). Samples from 30 benign meningiomas (32%) and three atypical tumors (12%) displayed Grade 3 staining, and only five benign tumors (5%) displayed Grade 2 staining. No Grade 0 or 1 stainings were noted. Normal cerebral cortex tissue stained for COX-2 displayed strong staining of the cytoplasm (Grade 4; Fig. 3C). Meningioma tissue samples were also stained for COX-1, 5-LO (Fig. 3F), and PTGER4. All 5-LO- and PTGER4-stained samples exhibited Grade 4 staining, whereas all corresponding COX-1 samples displayed Grade 2 staining. As a control, the antibodies to COX-2, 5-LO, and PTGER4 were treated with matching blocking peptides. The meningioma tissues showed no staining for these antibodies (Fig. 3E).

Immunocytochemical Analysis of Eicosanoids in Meningiomas

The immunocytochemical analysis for COX-1, COX-2, 5-LO, and PTGER4 was performed in the IOMM-Lee malignant meningioma cell line and in six meningiomas grown in cell culture. All seven specimens exhibited extensive cytoplasmic staining of COX-2, 5-LO, and PTGER4

(Grade 4; Fig. 4D–F), whereas cells stained with COX-1 displayed slightly weaker coloring (Grade 3; Fig. 4C). The COX-2, 5-LO, and PTGER4 antibodies were treated with matching blocking peptides. The benign meningioma samples showed no staining (Fig. 3E).

Expression of Eicosanoid Protein in Meningiomas

Twenty meningioma specimens, 84 meningioma primary cell cultures, the IOMM-Lee cell line, normal cerebral cortex and one surgical specimen of human dura were processed for Western blot experiments. Cytoplasmic and nuclear protein extracts were isolated. Both the COX-1 and -2 proteins have a molecular weight of approximately 70 kD. The molecular weight of 5-LO is approximately 78 kD, and the loading control β -actin has a molecular weight of 42 kD. All meningiomas sampled exhibited staining of a large band of approximately 70 kD, and 78 kD in the cytoplasm extracts (Fig. 5). None of the nuclear protein extracts showed staining for COX-1, COX-2, and 5-LO as expected by cytoplasmic proteins.

Expression of Eicosanoid RNA in Meningiomas

There was expression of eicosanoid RNA in 20 meningioma surgical specimens, 84 meningioma primary cell cultures, the IOMM-Lee cell line, normal cerebral cortex, and two dura surgical specimens, as shown on reverse-transcriptase PCR analysis. The PCR products of COX-1, COX-2, 5-LO, and PTGER4 were 143, 168, 207, and 167 bp, respectively, and the product of the loading control ACTB was 183 bp. The semiquantitative derived mean value of COX-1 is significantly lower than the other investigated eicosanoids (compared with COX-2; $p < 0.01$, t-test). No change of expression in high-grade meningiomas was noted. The expression of COX-2 is comparable to normal cerebral parenchyma and dura in benign and atypical meningiomas. The higher expression of COX-2 in WHO

FIG. 2. Graph showing a linear regression of the Ki 67 proliferation index compared with culture growth.

TABLE 1
*Immunoreactivity to COX-2 in 124 meningiomas stratified by WHO grade**

WHO Grade	Level of Cox-2 Reactivity					Total
	0	1	2	3	4	
I (benign)	0	0	5 (4)	30 (24)	60 (48)	95 (77)
II (atypical)	0	0	0	3 (2)	21 (17)	24 (19)
III (anaplastic)	0	0	0	0	5 (4)	5 (4)

* No. of patients (%)

Grade III meningiomas might be due to a bias based on few tissue samples. There are no significant differences in the expression of 5-LO in meningiomas and in normal cerebral cortex and dura. The mean value of PTGER4 is the same for normal cerebral cortex and dura as for WHO Grades I and II meningiomas (Fig. 6).

Discussion

Meningiomas are the most frequent benign tumors of the central nervous system. Although generally slow growing, these lesions continue to be a major cause of complications and death due to recurrence and unresponsiveness to adjuvant therapies. The finding that AA derivatives are expressed in meningiomas is fundamental and might create potential therapeutic opportunities.^{6,14,37,44} Few reports exist on the constitutive expression of these enzymes in healthy brain tissue, as this should be the baseline for tumor investigation.¹⁸ In the central nervous system, COX-2 is expressed in components such as neurons, glia, and cerebro-

vascular elements,^{2,19,28} or it can be induced by physiological or pathological stimuli.⁵² In accordance with the findings of Castelli and coworkers,⁶ who described similar expression of AA and metabolites in nine meningiomas and 10 healthy brain tissue specimens, we also found a high expression of AA derivatives in the healthy brain and in meningiomas of all grades. Ragel et al.⁴⁴ described an almost ubiquitous (95%) expression of COX-2 in 128 positively tested meningioma specimens. Because these researchers failed to compare their results with those from normal brain tissue, we validate their results adding data for healthy dura, normal brain tissue, and a substantial number of meningiomas of all different grades. Additionally, we verified the immunohistochemical findings through PCR, Western blot analysis, and immunocytochemistry, which should change the interpretation of previous reports proposing an upregulation of AA derivatives in meningiomas.³⁷ Although the investigated enzymes are not upregulated in meningiomas compared with normal brain and dura, future chemotherapeutic strategies could still have numerous starting points in attacking key molecules in tumor development.

Arachidonic acid metabolism via the COX and the LO pathways leads to the production of a number of metabolites that may modulate many of the mechanisms involved in tumor initiation, growth, and dissemination. Nathoo and associates³⁸ have demonstrated 5-LO expression solely in the cytoplasm of neurons in the healthy adult brain, whereas, in most astrocytoma surgical specimens, strong staining for 5-LO was seen in the nuclei of tumor cells. The authors argued that the activation of tumor cells may lead to the translocation of 5-LO to the nuclear membrane rather than the plasma membrane; furthermore, the nuclear import

FIG. 3. Photomicrographs demonstrating eicosanoid expression. Positive immunoreactivity appears red. A: Positive control (human tonsil tissue) with strong COX-2 immunoreactivity in the cytoplasm. B: Negative control (tonsil tissue). C: Normal cerebral cortex showing strong COX-2 reactivity in neurons. D–F: Benign meningioma tissue. Strong COX-2 immunoreactivity is noted diffusely throughout the cytoplasm (D), no staining after incubation with COX-2 antibody and COX-2 blocking peptide (E), and strong immunoreactivity to 5-LO is noted diffusely throughout the cytoplasm (F). NovaRED, original magnification $\times 10$.

FIG. 4. Photomicrographs demonstrating expression of COX-2 in a culture of meningioma primary cells. Positive immunoreactivity appears red. A: Strong COX-2 immunoreactivity is seen in the cytoplasm of the IOMM-Lee cell line, used as the positive control. B: Negative control (meningioma tissue without primary antibody treatment). C: Grade 3 immunoreactivity to COX-1 is shown. D–F: Grade 4 COX-2 (D), 5-LO (E), and PTGER4 (F) immunoreactivity is noted diffusely throughout the cytoplasm in meningioma. NovaRED, original magnification $\times 4$.

sequence of 5-LO protein has been identified in certain leukocytes.²² We found high cytoplasmic expression of 5-LO in almost all meningioma samples, with no expression in the nuclei of the tumor cells—either on immunohistochemical slides or on protein analysis. It remains to be determined whether 5-LO protein in meningiomas is functionally active and whether tumor cells express leukotriene receptors that could mediate cellular responses to 5-LO eicosanoids. Because 5-LO inhibitors are cytotoxic to cancer cell lines derived from several types of malignant tumors, further research into the potential use of 5-LO inhibitors for cancer prevention and treatment is warranted.^{1,15,16,38}

Staining for COX-1 had a significantly lower expression than for the other observed eicosanoids, in agreement with the results of the reverse-transcriptase PCR analysis. These observations correlate with the findings of Matsuo et al.,³² who reported a weak expression of COX-1 compared to COX-2 in normal human brain tissues, meningiomas, and other brain tumors. This could be due to the constitutive expression of COX-2 in the normal brain and particularly in meningiomas, leading to a takeover of functions elsewhere assigned to COX-1. Pai and colleagues⁴¹ reported that in addition to acting on its own receptor, PGE₂ could also activate the epidermal growth factor receptor, providing another potential mechanism for the tumor promotion effect of COX enzymes. Their results not only further support the role of COX-2 as a tumor promoter in the intestine, but also point to PGE₂ as the key mediator of the COX-2-related susceptibility to colon cancer. Wu and coworkers⁵¹ published a study demonstrating inhibition of prolifer-

ation and induction of apoptosis by celecoxib in human cholangiocarcinoma cells. Their results suggest that COX-2 plays a central role in the production of PGE₂ and that therefore specific inhibition with celecoxib, as a chemopreventive and chemotherapeutic agent of COX-2, inhibits proliferation and induces apoptosis of cells via suppression of PGE₂ production. This study for the first time demonstrated that the brain intrinsic prostaglandin receptor PTGER4 is strongly expressed in all meningioma specimens, pointing towards an important role of this enzyme in the development of meningiomas. Whether the compatible ligand of the receptor acts as a growth promotor or itself leads to molecular changes in the genetic program of the meningioma has to be elucidated.

As previously stated, COX-2 was expressed in all meningioma tissue samples. Because COX-2–derived metabolites may increase resistance to apoptosis, promote angiogenesis, and impair immune system surveillance, early prophylactic intervention with a COX-2 inhibitor may decrease tumorigenic progression in patients with malignant or invasive neoplasms. The tendency toward overexpression of COX-2 in more aggressive phenotypes of meningiomas suggests that COX-2 may play an important functional role in the later stages of invasive disease. Another hypothesis about increased COX-2 expression in more malignant meningiomas is that COX-2 levels could be an indicator of ischemia,³ supported by findings of necrotic areas within the tumor. Moreover, establishing a correlation between COX-2 expression and tumor recurrence or the Ki 67 proliferation index was not feasible, due to the overall high expression of COX-2 in meningiomas of

Fig. 5. Western blot analysis of three meningioma primary cells cultures, two meningioma surgical specimens, human dura, normal cerebral cortex, and IOMM-Lee cell line (numbered 1–8). Nuclear and cytoplasmic (cyto) protein extracts were isolated and probed with COX-1, COX-2, 5-LO, and beta-actin antibody (loading control). All nuclear protein extracts showed no staining, as expected by cytoplasmic proteins. Lanes 1–5: All meningioma primary cell cultures and the fresh surgical specimens exhibited strong staining for COX-1, COX-2, and 5-LO. Lane 6: Human dura tissue stained positively for COX-1, COX-2 and 5-LO, although the band of COX-2 was weaker. Lane 7: Normal human cerebral cortex tissue showing corresponding staining for COX-2 and 5-LO, and weaker staining for COX-1. Lane 8: The positive control IOMM-Lee cell line exhibited strong staining for COX-2 and 5-LO, and weak staining for COX-1.

all grades. Based on the results of our study, it is not possible to declare COX-2 a prognostic indicator for tumor grading—as has been proposed by Lin et al.²⁸ in meningiomas and Deininger and coworkers¹¹ in oligodendrogliomas—because no significant differences have been detected in eicosanoid expression among meningiomas of different WHO grades.

Radiation therapy has an important role in the management of recurrent meningiomas. In preclinical models of different cancers and clinical studies of gliomas, the addition of COX-2 inhibitors to radiotherapy can delay tumor regrowth compared with the use of either drugs or radiation alone.^{23,34,46,48} Pyo et al.⁴² found that in colorectal tumors expressing COX-2 that had been treated with radiation therapy and COX-2 inhibitors, there was a greater therapeutic response than to radiation therapy alone. Inhibition of COX-2 is augmented by the antiangiogenic effect of ionizing radiation through inhibition of nuclear factor- κ B.⁴ The use of COX-2 inhibitors with ionizing radiation is a

potentially attractive combination for recurrent meningioma.

In addition to the well-studied role of COX-2 in acute inflammatory processes, recent work clearly suggests that COX-2–derived metabolites contribute at multiple points in the early and late stages of carcinogenesis, including premalignant hyperproliferation, transformation, maintenance of tumor viability, growth, invasion, and metastatic spread.²⁴ We confirm that COX-2 might be a key player in a number of biological pathways leading not only to cancer in general, but also to meningiomas. Current evidence indicates that COX-2 promotes tumor-specific angiogenesis,^{27,31} inhibits apoptosis,⁵⁰ and induces proangiogenic factors such as vascular endothelial growth factor,^{29,47} inducible nitrogen oxide synthetase promoter,³³ and interleukin-6.⁵⁴ Taken together, the epidemiological data and preclinical studies in animal models have generated compelling interest in the potential use of COX-2 inhibitors in the prevention and chemotherapy of human tumors. Clinical trials are

FIG. 6. Results of a PCR analysis of COX-1, COX-2, PTGER4, and 5-LO in meningiomas, normal cerebral cortex, human dura tissue, and the IOMM-Lee cell line. The mean values were determined by semiquantitative analysis. Beta-actin was used to confirm equal loading among samples.

necessary to determine whether COX-2 inhibitors will provide clinical benefit, as well as to define the intervention points during tumor progression that will allow for optimal efficacy.

Conclusions

The promising effect of COX-2 inhibitors in anaplastic meningiomas were recently reported by Ragel and colleagues,⁴³ although the effective dosage was far higher than the recommended plasma levels using standard dosages in humans. The association of COX-2 and meningioma is unique and represents a potential area for therapeutic intervention with selective COX-2 inhibitors, either as an adjunct to or in combination with radiation therapy. With the results of this study we confirm the existence of multiple attacking points in the eicosanoid cascade for a powerful chemotherapeutic treatment in recurrent meningiomas. Further studies are on the way to understand the influence and importance of single factors in this enzyme network so that treatment options can be explored.

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