Cyclooxygenase-2 (COX-2) Overexpression in Meningiomas

Real Time PCR and Immunohistochemistry

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Abstract: Cyclooxygenase-2 (COX-2) is the inducible form of the enzyme involved in the first steps of the prostaglandins and thromboxane synthesis. COX-2 up-regulation is demonstrated in tumors where it can modulate tumoral progression, metastasis, multidrug resistance, and angiogenesis. Experimental data suggest a possible therapeutic use of the COX-inhibitors nonsteroidal antiinflammatory drugs (NSAIDs). NSAIDs can block tumor growth through many mechanisms, especially through antiangiogenic and proapoptotic effects. Moreover, NSAIDs can also improve the efficacy of radiotherapy, chemotherapy, and hormonal therapy. This study reviews the COX-2 expression as evaluated through immunohistochemistry and real time polymerase chain reaction (RT-PCR) in 23 meningiomas [14 World Health Organization (WHO) grade I; 5 WHO grade II; 3 WHO grade III; 1 oncocytic meningioma]. At immunohistochemistry all the lesions but 4 (83%) were COX-2 positive. At RT-PCR 9 meningiomas, 8 WHO grade I and 1 WHO grade II, showed a COX-2 expression greater than the reference value (average expression of all meningiomas that we studied). The association between tumor grade and immunohistochemical or RT-PCR COX-2 expression was not significant ($P = 0.427$ and $P = 0.251$, respectively). In conclusion, even if further studies on larger series are necessary, the common COX-2 overexpression in meningiomas may suggest considering the COX-2 inhibitors, alone or in combination with radiotherapy, a potential area of therapeutic intervention in some selected meningiomas.

Key Words: brain tumors, oncocytic meningioma, NSAIDs

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Cyclooxygenase (COX) is a membrane-bound enzyme involved in the first steps of the prostaglandins (PG) and thromboxane (TX) synthesis from arachidonic acid. There are 2 principal COX isoforms designated as COX-1 and COX-2, encoded by separate genes localized on chromosome 9q and chromosome 1q. Although COX-1, a 66 kd protein, is constitutively expressed almost ubiquitously in mammalian tissues, where it controls several normal physiological functions (ie, maintenance of the gastric mucosa, regulation of renal blood flow, platelet aggregation), COX-2, a 70 kd protein sharing 61% sequence identity with COX-1, is induced by several mitogenic and inflammatory stimuli.[1–9]

Lately, another COX isoform, known as COX-3, has been recognized. It derives through the retention of a highly structured G + C-rich intron 1 of the COX-1 gene and it is especially abundant in the cerebral cortex and in the heart.[10,11]

COX-2 plays a critical role in the development of neoplasms in various, although not completely clear, ways. Our knowledge on the role of COX-2 in the development of cancer is mainly derived from studies on colon carcinoma. Nevertheless, numerous researches have documented that COX-2 is also overexpressed in different premalignant and malignant conditions where it can stimulate gene transcription, tumoral growth, angiogenesis, metastasis, and immunosuppression, inhibit apoptosis, and cause resistance to chemotherapy through P-glicoprotein-170 overexpression. COX-2 overexpression may be a consequence of increased transcription and/or enhanced mRNA stability.[3,12–20]

Experimental data suggest a possible therapeutic use of the COX-inhibitors nonsteroidal antiinflammatory drugs (NSAIDs). NSAIDs can block tumor growth through many mechanisms, especially through antiangiogenic and proapoptotic effects. Moreover, NSAIDs can also improve the efficacy of radiotherapy, chemotherapy, and hormonal therapy.[21,22]

However, conventional NSAIDs (ie, aspirin, indomethacin, ibuprofen, etc), inhibiting both COX-2 and COX-1, elicit the suppression of PG production in the gastrointestinal and renal systems with the consequent possible damage of the gastric mucosa and of the renal and platelet functions. For this reason, there is a growing interest in new classes of NSAIDs that selectively inhibit COX-2. Nevertheless, some recent data seem to indicate the propensity of COX-2 inhibitors to trigger thrombo-embolic adverse events because of the inhibition of

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PGI_2 synthesis (PGI_2 mainly derives from endothelial COX-2 and causes vasodilatation, inhibition of platelet aggregation, and vascular smooth-muscle proliferation) unopposed by the inhibition of the TXA_2 synthesis (TXA_2 mainly derives from COX-1 and determines vasoconstriction, platelet aggregation, and vascular proliferation).[23,24]

COX-2 is frequently expressed in the brain during different pathologic conditions. There are numerous data showing the presence of COX-2 in the glioma-affected brain and indicating the therapeutic effectiveness of COX inhibitors in the gliomas.[2,3,15,19,20,25–30]

On the contrary, there is little reported information about the expression of COX-2 in meningiomas.[31–34]

In 2001, Matsuo et al[31] noted that all meningiomas that they immunohistochemically examined were COX-2 positive; in 2003, Lin et al[32] demonstrated that meningiomas with a more aggressive phenotype [as assessed by a modified 1993 World Health Organization (WHO) classification system] were associated with increasingly immunohistochemical COX-2 expression.

In the present work, we studied COX-2 expression through immunohistochemistry and real time polymerase chain reaction (RT-PCR) in a group of 23 meningiomas and we evaluated its possible correlation with tumor grade.

PATIENTS AND METHODS

Patients

Tissue specimens were obtained from 23 patients affected by meningiomas surgically treated at the Neurosurgical Service (Careggi Hospital, Florence, Italy) in which fresh tumoral tissue was available for RT-PCR. Eighteen (78%) were from women and 5 (22%) were from men. The average age at the time of the surgery was 52 years (range 29 to 76 years).

Twenty-two (96%) meningiomas were intracranial (1 of which was intraventricular) and the one remaining (4%) meningioma was spinal; 3 meningiomas (9%) were relapsed tumors and 2 meningiomas (9%) were multiple (Table 1).

RT-PCR

From each fresh surgical specimen, we selected a fragment macroscopically representative of the lesion. Successively, we cut each fragment in half: from one half, several 5-μm frozen sections stained with hematoxylin and eosin were obtained to verify the adequacy of the specimens selected for RT-PCR (presence of pathologic tissue only); the other half was immersed in RNA later (QIAGEN, Valencia, CA), kept overnight at +4°C and finally stored at −80°C until analyzed.

The thawed specimens were cut in small pieces and homogenized. After proteinase K digestion (250 μg/mL for 1 hour at 37°C), total RNA was isolated with 6100 Nucleic Acid PrepStation (manufacturer's protocol). Total RNA (500 ng) was subjected to reverse transcription of cDNA using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) (manufacturer's protocol). Real time quantitative PCR was performed on an ABI PRISM 7000 Sequence Detector System (Applied Biosystems). PCR products for COX-2 were detected using gene-specific primers and probes labeled with reporter day FAM (Assay on Demand, Applied Biosystems). Human glyceroaldehyde phosphate dehydrogenase (gAPDH) was used as endogenous control gene for normalization.

TABLE 1. Clinical Data, Histopathology, and Immunohistochemical and RT-PCR Results

	Clinical Data				Histopathology			
Case	Patient Sex/Age	Tumor			WHO Grade	Histotype	ICC	PCR
1	Female/38	Intracranial	Primary	Multiple	I	Fibrous	Diffuse ++	−1.01
2	Female/66	Intracranial	Primary	Single	I	Fibrous	Diffuse ++	−1.03
3	Female/46	Spinal	Primary	Single	I	Fibrous	Diffuse +	2.06
4	Male/29	Intracranial	Primary	Single	I	Fibrous	Diffuse ++	0.78
5	Female/41	Intracranial	Primary	Single	I	Fibrous	Negative	−0.76
6	Female/43	Intracranial	Primary	Multiple	I	Fibrous	Focal +	0.89
7	Female/35	Intracranial	Primary	Single	I	Fibrous	Diffuse ++	0.90
8	Female/68	Intracranial	Primary	Single	I	Fibrous	Negative	−0.71
9	Male/74	Intracranial	Primary	Single	I	Fibrous	Diffuse ++	0.39
10	Female/51	Intracranial	Primary	Single	I	Meningothelial	Diffuse ++	1.40
11	Female/32	Intracranial	Primary	Single	I	Meningothelial	Diffuse ++	1.81
12	Female/60	Intracranial	Primary	Single	I	Meningothelial	Negative	−0.88
13	Female/66	Intracranial	Primary	Single	I	Psammomatous	Focal +	−0.45
14	Male/64	Intracranial	Primary	Single	I	Angiomatous	Diffuse ++	2.50
15	Female/41	Intracranial	Primary	Single	II	Atypical	Focal +	−0.83
16	Female/76	Intraventricular	Primary	Single	II	Atypical	Diffuse +	0.14
17	Female/57	Intracranial	Primary	Single	II	Atypical	Diffuse ++	−0.29
18	Female/66	Intracranial	Primary	Single	II	Atypical	Negative	−1.07
19	Female/44	Intracranial	Relapse	Single	II	Chordoid	Diffuse +	−1.58
20	Male/69	Intracranial	Primary	Single	III	Anaplastic	Diffuse ++	−0.43
21	Male/37	Intracranial	Relapse	Single	III	Anaplastic	Diffuse ++	−0.48
22	Female/63	Intracranial	Relapse	Single	III	Anaplastic	Diffuse +	−0.92
23	Female/39	Intracranial	Primary	Single	—	Oncocytic	Focal +	−0.43

PCR reactions were carried out in 96-well plates with 20 μL per well using 1 × TaqMan Universal PCR MasterMix. After incubation for 2 minutes at 50°C and 10 minutes at 95°C, the reaction continued for 50 cycles at 95°C for 15 seconds and 60°C for 1 minute. The $2^{-\Delta\Delta Ct}$ method described by Livak et al[35] was used to calculate fold expression levels relative to the average value of all the meningiomas RNA specimens (the calibrator). We chose the average expression of all the meningiomas as the reference value to emphasize the strong difference of expression.

Histopathology and Immunohistochemistry

After drawing off the small samples for RT-PCR, the remaining tissues were routinely fixed in 10% buffered formalin and embedded in paraffin.

Five-μm thick sections were stained with hematoxylin and eosin for morphological evaluation. The diagnostic criteria we used were those indicated by the most recently revised WHO classification of tumors of the nervous system[36].

Further 5-μm thick sections of the most representative specimen of each case were mounted on electrostatic slides for the immunohistochemical evaluation of COX-2 expression (clone COX 229; Zymed Laboratories, San Francisco, CA). Paraffin and antigen retrieval were performed by immersing the slides in boiling Tris-EDTA-Citrate ready-to-use buffer (W-cap-"wax capture"-Tris-EDTA-Citrate buffer pH 8, BIO-OPTICA) for 20 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in distilled water for 10 minutes. The primary antibody was used at 1:50 dilution at room temperature for 1 hour, followed by incubation with peroxidase conjugate polymer (Chemmate Dako Envision Detection Kit Peroxidase DAB rabbit-mouse) for 30 minutes. The reaction products were visualized with diaminobenzidine (Chemmate DAB Chromogen Dako) for 5 minutes. The nuclei were counterstained with hematoxylin. The following procedures were used as negative controls:

- a nonimmune serum in place of the primary antibody; and
- omitting the primary antibody.

An ascertained COX-2-positive colon adenocarcinoma was used as positive control.

Cytoplasmic COX-2 expression was considered as negative when it was less than 25% or absent, as focal when it was present in more than 25% and less than 50% of the neoplastic cells, and as diffuse when it was present in 50% or more of the neoplastic cells. Furthermore, we graded immunocoloration as + (weak) or ++ (intense) on the basis of intensity of staining.

Statistical Analysis

The shift of expression level of COX-2 as estimated through immunohistochemistry and relative RT-PCR was calculated according to the Wilcoxon Mann-Whitney P value ≤ 0.05 was considered to be statistically significant.

RESULTS

Fourteen (61%) lesions were WHO grade I meningiomas (9 fibrous, 3 meningothelial, 1 psammomatous, 1 angiomatous); 5 (22%) were WHO grade II meningiomas (4 atypical, 1 chordoid); 3 (13%) were anaplastic meningiomas; and the one remaining (4%) was oncocytic meningioma, a novel uncategorized rare variant showing oncocytic differentiation (wide granular cytoplasm full of numerous swollen mitochondria) and uncertain prognosis (Fig. 1).

At immunohistochemistry all the lesions but 4 (2 fibrous, 1 meningothelial, 1 atypical) (83%) were COX-2 positive. Eleven positive meningiomas were scored as diffuse ++ (5 fibrous, 2 meningothelial, 1 angiomatous, 1 atypical, 2 anaplastic), 4 as diffuse + (1 fibrous, 1 atypical, 1 chordoid, 1 anaplastic), and 4 as focal + (1 fibrous, 1 psammomatous, 1 atypical, 1 oncocytic). Endothelial cells, in either positive or negative meningiomas, were COX-2 positive (Fig. 2).

There were no significant differences in immunohistochemical expression of COX-2 related to histological grade ($P = 0.427$).

RNA extraction was successful in all cases. The COX-2 expression of each meningioma was compared with the average value of expression (reference value) of the entire group of meningiomas (23 cases). Nine meningiomas (39%), 8 WHO I (5 fibrous, 2 meningothelial, and 1 angiomatous) and 1 WHO II, showed COX-2 expression greater than the average of the entire group of meningiomas. Angiomatous meningiomas had the highest COX-2 expression (Figs. 3, 4).

There were no significant differences in RT-PCR expression of COX-2 related to histologic grade ($P = 0.251$).

When comparing the RT-PCR results with the immunohistochemical results, there was significant discordance in 5 out of 23 (22%) meningiomas. Precisely,

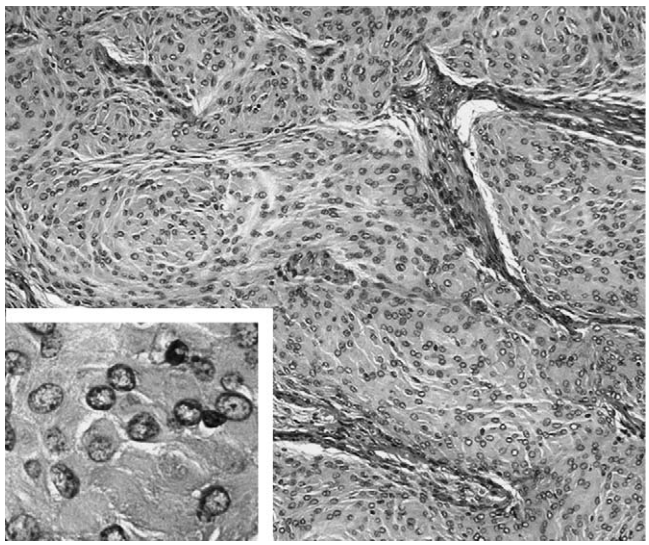

FIGURE 1. Oncocytic meningioma: the lesion is composed of sheets of rounded cells with wide granular eosinophilic cytoplasm (inset).

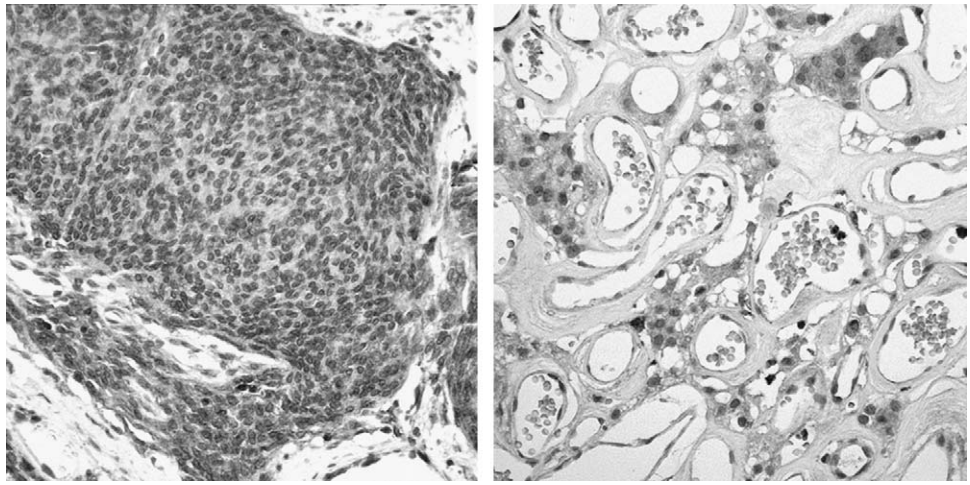

FIGURE 2. Immunohistochemistry: meningothelial (right) and angiomatous (left) positive meningiomas (neoplastic and endothelial cells).

5 meningiomas (2 fibrous, 1 atypical, 2 anaplastic) were diffusely and intensely positive at immunohistochemistry (diffuse ++ score) and showed low (less than the reference value) COX-2 expression at RT-PCR. In the remaining cases, the lesions were negative or weakly positive at immunohistochemistry (diffuse + or focal + score) and presented low (less than the reference value) COX-2 expression at RT-PCR, whereas those showing diffuse and intense (diffuse ++ score) COX-2 immunoreaction had high (more than the reference value) COX-2 expression at RT-PCR.

DISCUSSION

Meningiomas are frequent neoplasms (13% to 26% of primary intracranial tumor) arising from the leptomeningeal covering of the brain and spinal cord. They typically manifest in adult women. Although meningiomas are generally considered slow-growing, benign tumors, their long-term prognosis may be tainted by recurrences or by aggressive behavior (invasion of the brain and adjacent bones).[36,37]

The morphology of meningiomas is highly polymorphic, with numerous classified subtypes (the most

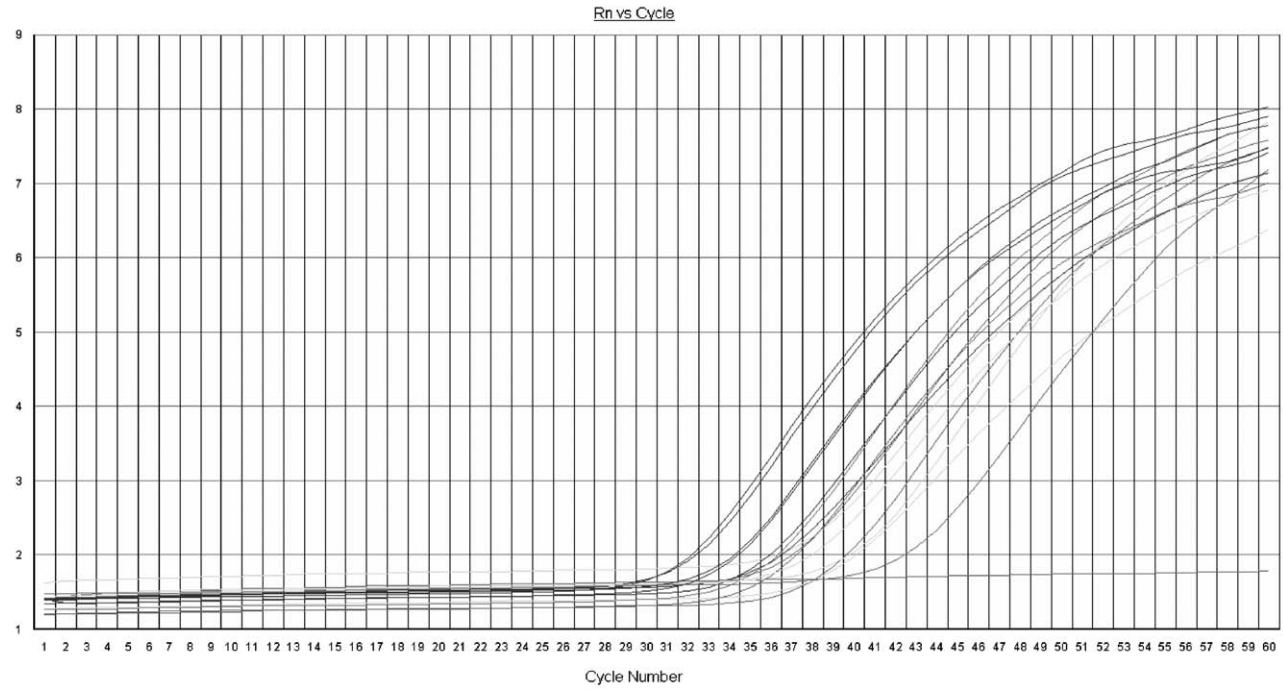

FIGURE 3. RT-PCR: the graph shows fluorescence signals accumulated at different PCR cycles.

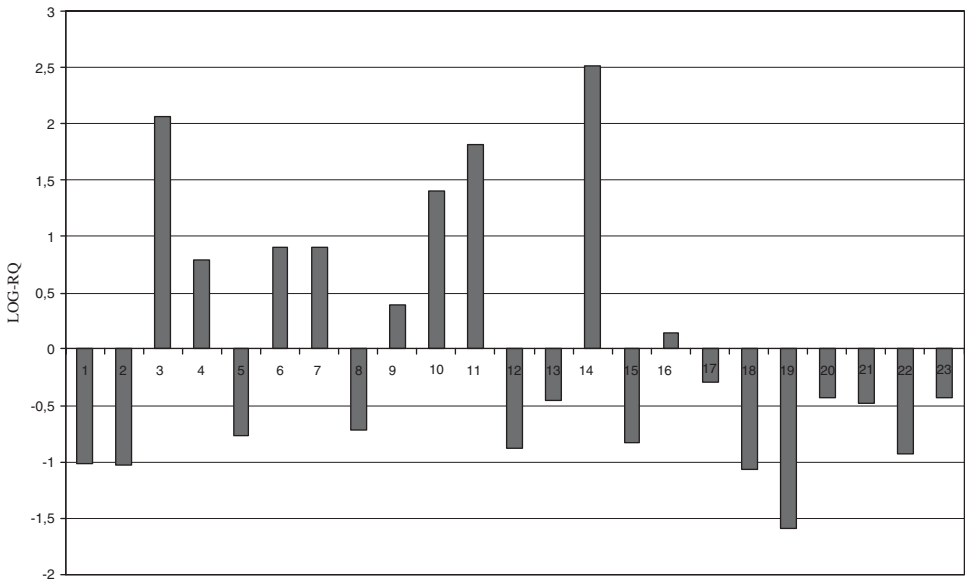

FIGURE 4. Relative expression of COX-2 in different types of meningioma. The average value of all the meningiomas RNA specimens was used as a reference value; cases 1 to 9: fibrous meningiomas; cases 10 to 12: meningothelial meningiomas; case 13: psammomatous meningioma; case 14: angiomatous meningioma; cases 15 to 18: atypical meningiomas; case 19: chordoid meningioma; cases 20 to 22: anaplastic meningiomas; case 23: oncocytic meningioma.

recent WHO scheme recognizes 13 variants), several uncategorized histologic variants (ie, oncocytic meningioma), and 3 histologic grades (WHO I; WHO II; WHO III) depending upon the presence of histopathologic features thought to predict unfavorable behavior (mitosis, increased cellularity, small cells, macronucleoli, sheetlike growth, necrosis, and sarcomatous, carcinomatous or melanomalike appearance).[36–38]

In the majority of cases, chordoid and clear cell that are equalized to atypical meningiomas, and rhabdoid and papillary that are equalized to anaplastic meningiomas, the histologic variant does not condition the prognosis.[36]

The clinical behavior of meningioma mainly depends upon the extent of resection and histologic grade. The overall recurrence rate of meningiomas is reported to be approximately 20%, with higher rates for partially excised meningiomas (30% to 40%), atypical meningiomas (38%), and malignant meningiomas (78%).[36,37]

The management of certain meningiomas such as multiple and recurrent lesions, in which total resection is difficult to obtain (ie, meningiomas of the skull base or involving the dural venous sinuses), and those in patients who are medically unsuitable for surgery, may be difficult. If surgical excision, the cornerstone of treatment for all types of meningiomas, is not possible, radiation therapy is beneficial whereas stereotactic radiation, interstitial brachytherapy, hormonal manipulation or chemotherapy may represent therapeutic opportunities in some selected patients.[39–43]

As indicated by numerous data concerning colon carcinoma, NSAIDs may be considered interesting therapeutic opportunities in the treatment of different tumors. In fact, the demonstration that NSAIDs significantly reduce the risk of insurgence of colorectal tumors and the number of preexisting adenomas in adenomatous familial syndrome-affected patients suggested the possibility of an analogous role in other tumors, and several reports have confirmed this hypothesis.[21,22,44–50]

The possibility of a therapeutic use of NSAIDs in meningiomas can be argued if COX-2 overexpression is proved.

In accordance with Matsuo et al,[31] we noted COX-2 overexpression in the majority of meningiomas. On the other hand, contrary to the results obtained by Lin et al[32] using a modified 1993 WHO grading system, increased COX-2 expression was not detected in high-grade meningiomas with respect to low-grade meningiomas. Actually, Lin himself supposed that increased COX-2 expression in more malignant meningiomas could be interpreted as an indicator of ischemia (COX-2 is elevated around areas of necrosis and high-grade meningiomas present necrosis more often than low-grade meningiomas) rather than as a marker of malignancy. When evaluated through RT-PCR, increased COX-2 expression may also be related to vessel density of the lesion (endothelial cells overexpress COX-2). Angiomatous meningiomas of our series showed the highest COX-2 expression at RT-PCR.

The discordant results between immunohistochemistry and RT-PCR could be explained by the modality of COX-2 quantification (our reference value was the average expression of all 23 meningiomas) or by the fortuitous evaluation of areas with different levels of COX-2 expression by one method with respect to another.

In conclusion, we documented that meningiomas, independent of tumor grade, commonly overexpress COX-2. This result, even though further studies on larger and homogeneous series are certainly necessary, could suggest evaluating the possibility of utilization of NSAIDs, alone or in combination with radiotherapy, in the treatment of some selected meningiomas.

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