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Cyclooxygenase-2 Expression in Human Gliomas: Prognostic Significance and Molecular Correlations[1]

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ABSTRACT

Cyclooxygenase (COX)-2, the inducible isoform of prostaglandin H synthase, has been implicated in the growth and progression of a variety of human cancers. Although COX-2 overexpression has been observed in human gliomas, the prognostic or clinical relevance of this overexpression has not been investigated to date. In addition, no study has analyzed the relationship between COX-2 expression and other molecular alterations in gliomas. Consequently, we examined COX-2 expression by immunohistochemistry in tumor specimens from 66 patients with low- and high-grade astrocytomas and correlated the percentage of COX-2 expression with patient survival. We also analyzed the relative importance of COX-2 expression in comparison with other clinicopathological features (age and tumor grade) and other molecular alterations commonly found in gliomas (high MIB-1 level, p53 alteration, loss of retinoblastoma (Rb) protein or p16, and high bcl-2 level). Kaplan-Meier analyses demonstrated that high COX-2 expression (>50% of cells stained positive) correlated with poor survival for the study group as a whole ($P < 0.0001$) and for those with glioblastoma multiforme in particular ($P < 0.03$). Cox regression analyses demonstrated that COX-2 expression was the strongest predictor of outcome, independent of all other variables. In addition, high COX-2 expression correlated with increasing histological grade but did not correlate with positive p53 immunostaining, bcl-2 expression, loss of p16 or retinoblastoma protein expression, or high MIB-1 expression. These findings indicate that high COX-2 expression in tumor cells is associated with clinically more aggressive gliomas and is a strong predictor of poor survival.

INTRODUCTION

PGHS[3] or COX is the rate-limiting enzyme in the synthesis of PGs from arachidonic acid (1, 2). PGHS is a bifunctional enzyme containing a COX site that converts arachidonic acid to PGG2 and a peroxidase site that reduces PGG2 to PGH2. PGHS is the primary target of aspirin and nonsteroidal anti-inflammatory drugs (3). Two isoforms of PGHS, referred to as COX-1 and COX-2, have been described. Although both isoforms catalyze the same enzymatic reactions and have similar K_m and V_{max} values for arachidonic acid, significant differences exist between them (4). The genes for each isoform are located on separate chromosomes (9q for COX-1 and 1p for COX-2). Moreover, the active site of COX-2 is larger than that for COX-1. COX-2 is concentrated in the nuclear envelope, whereas COX-1 localizes primarily to the endoplasmic reticulum. Glucocorticoids inhibit COX-2 expression but not COX-1 expression. Most importantly, COX-1 is constitutively expressed in nearly all normal tissues and mediates the synthesis of PGs required for physiological tissue homeostasis. In contrast, COX-2 expression is inducible and increases in response to various stimuli, including inflammatory signals, mitogens, cytokines, and growth factors (4–6).

Accumulating evidence suggests that increased PG levels via overexpression of the inducible COX-2 isoform are important in the development of human cancer (7). Increased PG synthesis has been shown in a variety of tumor types (8–11), and recent studies have demonstrated increased COX-2 expression in pancreatic, gastric, esophageal, prostate, lung, and head and neck cancers (10, 12–29). The role of COX-2 has been best worked out in colorectal cancer, where adenomatous polyps and malignant colonic tumors have been shown to express higher levels of COX-2 than does normal intestinal mucosa, which has low to undetectable COX-2 expression (17, 18, 24, 25). Clinical trials have demonstrated that nonsteroidal anti-inflammatory drugs, which can inhibit both COX-1 and COX-2, significantly reduce the number of preexisting adenomas in patients with familial adenomatous polyposis (30) and can reduce the risk of sporadic colorectal cancer (31, 32). Furthermore, in several transgenic and carcinogen-induced colon tumor animal models, COX-2 levels have been shown to be high in intestinal polyps, and inactivation of COX-2 by specific inhibitory drugs or by genetic crosses with COX-2 null mice reduces or prevents tumor formation (22, 33–37). Thus, significant evidence supports the role of COX-2 in several cancers and the use of COX-2 inhibitors in the prevention and treatment of these cancers.

In addition to biological and clinical evidence, COX-2 has been implicated at the molecular level in several tumor-related processes. Importantly, the tumor suppressor p53, which is mutated or inactivated in most human cancers and plays a role in the radio- and chemosensitivity of tumors, may inhibit COX-2 expression (38). Furthermore, increased COX-2 expression has been associated with increased amounts of the antiapoptotic protein bcl-2 (39). *In vitro* studies have demonstrated that COX-2 may stimulate angiogenesis (40–42), a critical step in the progression of neoplasms to more aggressive grades and in the development of metastases. Lastly, COX-2 may regulate the invasive potential of tumor cells (43). Thus, COX-2-mediated pathways may underlie several phenotypic features of gliomas by interacting with other tumor-related oncoproteins (39, 44, 45).

Malignant gliomas are the most common tumors of the central nervous system. Despite the use of surgery, radiation, and conventional chemotherapy, the median survival time for patients with GBM, the most malignant type of glioma, is only 1 year (46). Many GBMs are believed to arise by progression from low-grade astrocytomas (WHO grade 2) and anaplastic astrocytomas (WHO grade 3). The molecular mechanisms underlying the malignant phenotype and progression are beginning to be understood and appear at least in part to be associated with inactivation of tumor suppressor genes (including *p53*, Rb, *p16/INK4A*, and *MMAC*) and overexpression of oncogenes [*ras*, epidermal growth factor receptor, and *Akt*] (47, 48).

The importance of PGs and COX-2 in the formation and progression of gliomas was suggested by early reports that described increased levels of PGs within gliomas and correlations between increased PG synthesis and tumor grade (11, 23). Based on these findings, a small number of reports (49, 50) have described the role of

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[3] The abbreviations used are: PGHS, prostaglandin H synthase; PG, prostaglandin; COX, cyclooxygenase; GBM, glioblastoma multiforme; Rb, retinoblastoma.

COX-2 expression in human gliomas. Deininger *et al.* (49) initially reported that COX-2 expression (measured by immunohistochemistry) typically occurred in less than 20% of glioma cells within a given specimen. In contrast, Joki *et al.* (50) recently demonstrated with Western blotting that COX-2 was expressed in all glioma specimens tested; moreover, the percentage of positively stained cells by immunohistochemistry was significantly higher in high-grade gliomas than in low-grade gliomas or normal brain. In that study, 96% (24 of 25) of the GBM specimens stained had more than 50% of the cells staining positively. These authors also demonstrated that the proliferation and invasion of cultured glioma cell lines could be inhibited *in vitro* by the specific COX-2 inhibitor NS-398, an observation supporting a functional role for COX-2 in glioma.

To further evaluate the role of COX-2 in human gliomas, we hypothesized that if COX-2 was contributing to the malignant phenotype of gliomas, then elevated COX-2 expression should correlate with tumor grade and should be predictive of shortened survival independent of other clinical and molecular variables. Although recent studies have reported significant relationships between shortened patient survival and elevated COX-2 expression in colorectal, liver, and lung cancers (27–29), to our knowledge, such a relationship between COX-2 expression and patient survival in gliomas has not been determined. Therefore, in an effort to determine its prognostic significance, we examined COX-2 expression by immunohistochemistry in tumor specimens from 66 patients with low- and high-grade astrocytomas and correlated the extent of COX-2 staining with patient survival. Moreover, although studies have suggested interactions between COX-2 and p53 or bcl-2 in nonglial tumors, the relationship between COX-2 expression and a variety of other molecular markers of tumor progression has not been reported for gliomas. Thus, in an effort to examine the relationship between COX-2 expression and other tumor-related proteins, we also analyzed the relative importance of COX-2 expression compared with that of other molecular alterations found in gliomas, including MIB-1 staining (as an indicator of mitotic rate), p53 alteration, loss of Rb or p16, and high bcl-2 levels.

We found that the extent of COX-2 staining (measured as a percentage of positively stained tumor cells) was an independent predictor of patient survival for both the overall sample group and patients with GBM. Moreover, high COX-2 expression correlated with high histological grade but not with other molecular markers of tumor aggressiveness, including expression of p53 or overexpression of bcl-2.

MATERIALS AND METHODS

Clinical Data and Tumor Specimen Acquisition. All patients were treated at The University of Texas M. D. Anderson Cancer Center between 1980 and 1996. Clinical data were obtained by retrospective chart review. Survival was determined from the date of initial surgery. Follow-up was available for all patients.

Tumor specimens were obtained by surgical resection in all cases and fixed in 10% formalin before being processed in paraffin. Histological diagnosis and tumor grading were verified by rereview in all cases. Most of the tumor specimens had been analyzed previously for the degree of proliferation with a MIB-1 antibody. Most cases had also been assessed by immunohistochemistry for the presence of p53, p16, bcl-2, and Rb proteins. With the exception of the results for bcl-2 and MIB-1, the results of these studies have been reported elsewhere (51–61).

Evaluation of COX-2 Expression. COX-2 expression was determined by immunohistochemical staining with a polyclonal rabbit antihuman COX-2 antibody (Oxford Biomedical Research, Oxford, MI). Before brain tumor specimens were analyzed, this antibody was tested at various dilutions with a colon carcinoma specimen known to contain high COX-2 levels to identify the optimal antibody concentration required for reproducible immunohistological staining with minimum background staining. The COX-2 antibody was also prewashed with COX-2 protein, which was found to eliminate staining.

Four-μm-thick sections were cut from each specimen for COX-2 staining. Paraffin sections were prewarmed to 60°C, deparaffinized in two changes of xylene, rinsed in decreasing ethanol concentrations (100% to 70%), and rehydrated in PBS. Endogenous peroxidase was inactivated with 0.3% H_2O_2 in methanol, and the slides were incubated overnight with the COX-2 antibody at a 1:100 dilution. The slides were then rinsed with PBS and incubated with an avidin-conjugated horse antirabbit antibody for 30 min. After further rinsing with PBS, the slides were treated with a solution of biotinylated peroxidase and visualized by using the AEC Substrate Kit (Vector Laboratories, Burlingame, CA). Nonimmunized rabbit IgG (Vector Laboratories) was used as a negative control, and sections of colon cancer known to express COX-2 protein were used as a positive control.

Immunohistochemically stained slides were reviewed by all of the authors. The intensity of staining was determined and recorded as strong, weak, or negative. The type of cells stained (*e.g.*, malignant astrocytes, reactive astrocytes, neurons, and endothelial cells) was determined. The distribution of cell staining (near or far from necrotic areas) was also assessed. Most importantly, the percentage of cells expressing COX-2 was estimated by dividing the number of positively stained astrocytic tumor cells by the total number of tumor cells per high-power field. Tumor sections were grouped according to extent of COX-2 positivity as 0–25%, 26–50%, 51–75%, or 76–100% positive.

Other Molecular Markers. The same tumor specimens were analyzed by immunohistochemistry with antibodies to p53 (PAb 1801; Oncogene Science), p16 (C-20; Santa Cruz Biotechnology), Rb (G3-245; PharMingen), and bcl-2 (124; Dako). The details of methods from p53, p16, and Rb analysis have been reported previously (51–61). The method of bcl-2 staining was the same as that used for the other antibodies (dilution, 1:100). Tumor specimens were analyzed, and grades for each marker were given as defined in the footnotes of Table 1. Proliferation rates were determined by staining with a MIB-1 antibody (Immunotech) that recognized the Ki-67 antigen according to the method of Krishna *et al.* (59).

Statistical Analysis. Statistical analyses were performed by using the SPSS for Windows Version 10.0.7 software package (SPSS Inc., Chicago, IL). Survival time was estimated in months from the date of histological diagnosis (when the specimen was obtained) to the date of death or the date of last follow-up (for the patients who were still alive at the time of the study).

Table 1 *Correlation of COX-2 expression with clinical, pathological, and molecular features of GBM*

	Percentage of tumor cells staining for COX-2		
	<50%	>50%	*P*
Patient age, mean	44 years	41 years	0.556
Tumor histology			
Low-grade astrocytoma	70%	30%	0.031[a]
Anaplastic astrocytoma	56%	44%	
GBM	29%	71%	
MIB-1 immunostaining			
0–15%	54%	46%	0.188[b]
15–30%	44%	56%	
>30%	20%	80%	
p53 immunostaining[c]			
Grade 0, 1, or 2 (<30%)	50%	50%	0.196[b]
Grade 3 (>30%)	25%	75%	
Rb immunostaining[d]			
Grade 0–1	35%	65%	0.402[b]
Grade 2–5	47%	53%	
p16 immunostaining			
0	55%	45%	0.054[b]
1	20%	74%	
Bcl-2 immunostaining[e]			
0 or 1 (<30%)	43%	57%	0.688[a]
2 (>30%)	29%	71%	

[a] Pearson χ^2 test (asymptotic significance, two-sided).
[b] Fisher's exact test (two-sided).
[c] Grading system for p53 (nuclear) and bcl-2 (cytoplasmic) immunostaining: 0, none; 1, <5%; 2, 6–30%; and 3, >30%.
[d] Grading system for Rb immunostaining: 0, none; 1, 1–5%; 2, 6–40%; 3, 41–60%; 4, 61–90%; and 5, >90%.
[e] Grading system for p16 immunostaining: 0, none; and 1, + (any).

Survival was plotted, and median survival time was estimated by the Kaplan-Meier method. The survival times and the strength of associations between categories within a variable or between variables were compared with the log-rank test. Variable effects on the time of survival were investigated with a stepwise Cox regression analysis. Ten variables were analyzed: (*a*) age; (*b*) histological grade; (*c*) extent of COX-2 staining; (*d*) COX-2 nuclear staining; (*e*) COX-2 endothelial cell staining; and (*f*) MIB-1, p53, Rb, p16, and bcl-2 staining levels. Statistical significance was defined as a probability value less than 0.05. Associations between COX-2 and histological grade and the molecular markers were determined by using the χ^2 test.

RESULTS

Tumor specimens from 66 patients with astrocytic gliomas were analyzed. The mean age at diagnosis was 42 years (age range, 2–78 years). All patients had Karnofsky performance scores of at least 70 at diagnosis. Patients were treated with surgery and adjuvant radiation therapy for all patients with GBM or anaplastic astrocytoma and for most patients with low-grade astrocytoma. Most patients also were given a variety of chemotherapeutic agents. No attempt was made to standardize therapy. There were 31 GBMs (47%), 25 anaplastic astrocytomas (38%), and 10 low-grade fibrillary astrocytomas (15%).

Qualitative Immunohistochemical Analysis for COX-2. COX-2 immunoreactivity was observed in all of the brain tumor specimens examined. In sections containing areas of normal cortex adjacent to or infiltrated by neoplastic astrocytes, COX-2 staining was observed in the cytoplasm of normal neurons and rarely observed in normal endothelial cells. In cases in which reactive astrocytes (defined as cells with regular nuclei and symmetric processes) were present, intense COX-2 cytoplasmic staining was evident (Fig. 1*A*). Within tumor cells, most of the COX-2 immunoreactivity was observed in the cytoplasm, as reported previously (49, 50). However, nuclear staining was seen in 10 cases of GBM and 6 cases of anaplastic astrocytoma (Fig. 1*B*). Although COX-2 staining was evident in cells located away from necrotic areas, accumulation of COX-2-positive tumor cells was particularly conspicuous around areas of necrosis within pseudopalisade cells (Fig. 1*C*).

Correlation of COX-2 Expression with Clinical, Pathological, and Molecular Markers. COX-2 expression did not correlate with patient age (Table 1). However, the extent of COX-2 staining was greater in GBMs than in low-grade astrocytomas (Table 1). Among GBM specimens, 71% had more than 50% COX-2-immunopositive tumor cells, compared with 44% of anaplastic astrocytomas and 30% of low-grade astrocytomas. Similarly, only 3% of GBMs had fewer than 25% COX-2-positive cells, as compared with 12% of anaplastic astrocytomas and 40% of low-grade astrocytomas. Thus, the extent of COX-2 staining increased with glioma grade, and this finding was statistically significant.

The extent of COX-2 staining did not correlate statistically with any of the molecular markers tested (Table 1), although COX-2 expression tended to correlate with p16 immunostaining ($P < 0.054$), with tumors showing high COX-2 expression being more likely to demonstrate p16 staining than tumors with low COX-2 immunostaining. However, high COX-2 expression did not correlate statistically with alterations in p53 protein (*i.e.*, positive p53 immunostaining) or with high bcl-2 immunoreactivity, although there was a trend for tumors with a high percentage of COX-2 staining to demonstrate higher frequency of p53 immunoreactivity and bcl-2 (Table 1). Tumors with high mitotic rates tended to have high COX-2 staining.

COX-2 Expression Level and Patient Survival. Kaplan-Meier survival plots for all patients showed a statistically significant association between high levels of COX-2 expression and poor outcome ($P < 0.0001$; Fig. 2*A*). Because survival of patients with brain tumors has been associated with several clinicopathological variables, we attempted to define the relative contribution of COX-2 expression to survival by using multivariate (Cox regression) analyses with 10 variables (age, histology, extent of COX-2 staining, COX-2 nuclear staining, COX-2 endothelial staining, and MIB-1, p53, Rb, p16, and bcl-2 staining). In the initial univariate analysis, age ($P = 0.01$), tumor

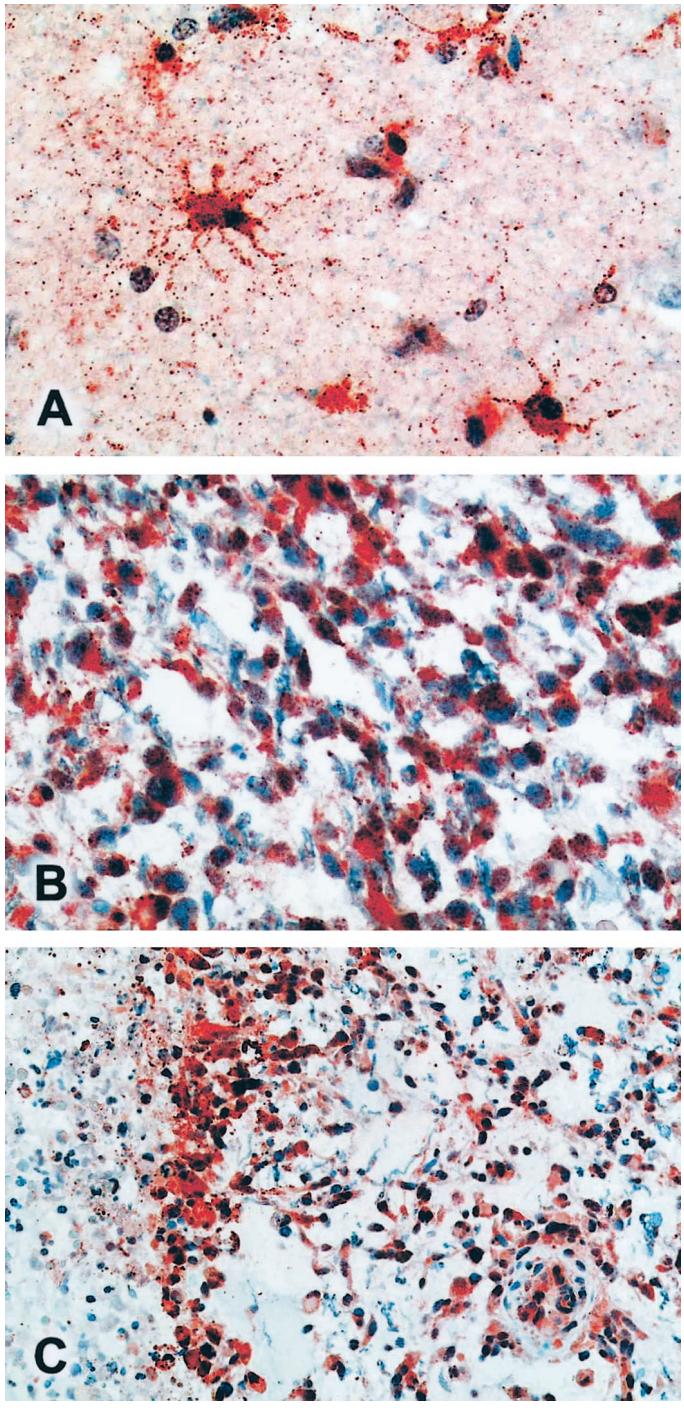

Fig. 1. *A*, reactive astrocytes in brain tissue adjacent to tumor show numerous branching cytoplasmic processes with strong immunoreactivity for COX-2. Immunohistochemistry for COX-2 was assessed using the AEC Substrate Kit for detection and a hematoxylin counterstain (×400). *B*, anaplastic astrocytoma with immunoreactivity in both nuclei and cytoplasm of neoplastic cells for COX-2. Immunohistochemistry for COX-2 was assessed using the AEC Substrate Kit for detection and a hematoxylin counterstain (×400). *C*, accumulation of immunoreactivity for COX-2 in the cytoplasm of neoplastic astrocytes at the interface between an area of necrosis (*left*) and tumor (*right*). This is a zone of pseudo-palisading around an area of tumor necrosis. Immunohistochemistry for COX-2 was assessed using the AEC Substrate Kit for detection and a hematoxylin counterstain (×200).

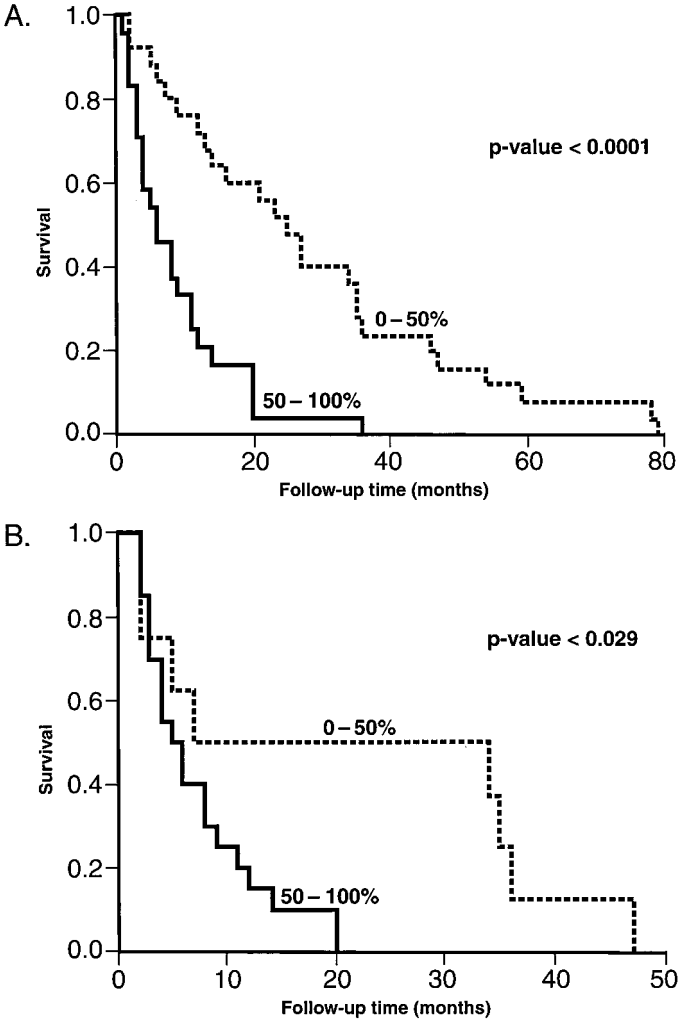

Fig. 2. *A*, actuarial survival (Kaplan-Meier method) of patients with malignant glioma whose tumors had greater than or less than 50% COX-2 immunostaining. *B*, actuarial survival of patients with GBM based on the percentage of COX-2 staining.

grade ($P = 0.001$), and extent of COX-2 staining ($P < 0.001$) were all significant (Table 2). For the multivariate analysis, we used the backward stepwise (Wald) method, in which variables were removed at each step, based on a 0.05 level of significance. The last three variables to be removed were age, histological grade, and COX-2 expression (Table 3). Only COX-2 expression was present at the final step ($P < 0.001$), indicating that COX-2 expression has a major impact on survival rate. High COX-2 expression has a relative risk of 0.185, with a 95% confidence interval of 0.076–0.447. Therefore, patients with tumors in which more than 50% of cells stain for COX-2 have a worse survival rate than patients with tumors in which fewer cells express COX-2.

Although the above-mentioned analysis suggests otherwise, the data demonstrating an association between COX-2 expression and survival could reflect differences between survival rates for patients with different tumor grades. To evaluate the effect of high COX-2 expression within tumor grades, we analyzed the GBM subgroup for an association between COX-2 expression and survival. We found that GBM patients with tumors having a high percentage of COX-2 expression had a statistically significant poorer prognosis than did those with tumors having a low percentage of COX-2 (Fig. 2*B*). In the anaplastic astrocytoma and low-grade astrocytoma groups, no difference in survival was found as a function of COX-2 expression, but the numbers of patients in these groups were too small for accurate statistical sampling. Therefore, at least within GBM, COX-2 expression seems to be predictive of survival.

DISCUSSION

We have demonstrated that increasing tumor grade correlates with a high percentage of tumor cells expressing COX-2 and that high COX-2 staining predicts a poor prognosis in patients with astrocytic brain tumors in general and in patients with GBM in particular. However, high COX-2 expression did not correlate with p53 or bcl-2 immunostaining, Rb or p16 expression, or MIB-1 staining. Taken together, these observations support the hypothesis that COX-2 expression is an important determinant of the malignant phenotype of gliomas, but the mechanism by which this occurs remains unclear.

Consistent with the findings of others (49, 50), we observed significant COX-2 staining within astrocytic tumor cells located predominantly but not exclusively around areas of necrosis. This distribution may represent induction of COX-2 by hypoxia or hypoglucosemia, as has been observed for ischemia (62) and trauma (63), and is consistent with the inducibility of COX-2. However, we also observed COX-2 staining at sites without necrosis, suggesting that COX-2 may be constitutively expressed in tumor cells. The possibility of unregulated COX-2 expression is supported by *in vitro* studies demonstrating constitutive COX-2 expression in cells cultured in supplemented media and normoxia (50, 64, 65). We also observed COX-2 staining

Table 2 *Univariate COX regression analysis of possible contributions to survival*

Variable	Relative risk (95% CI)[a]	*P* (log-rank)
Age (yrs)		0.010[b]
0–29	0.367 (0.164–0.821)	0.015
30–39	0.326 (0.156–0.680)	0.003
40–59	0.783 (0.355–1.726)	0.544
60+[c]		
Tumor histology		0.001[b]
Low-grade astrocytoma	0.157 (0.057–0.433)	<0.001
Anaplastic astrocytoma	0.459 (0.247–0.851)	0.013
Glioblastoma[c]		
Level of COX-2 expression		<0.001[b]
0–50%	0.279 (0.145–0.535)	<0.001
50–100%[c]		
COX-2 nuclear staining		0.253[b]
Negative	1.498 (0.749–2.995)	0.253
Positive[c]		
COX-2 vessel staining		
Positive		
Negative		
MIB-1 staining		0.074[b]
0–5	0.470 (0.201–1.095)	0.080
5–15	0.461 (0.170–1.249)	0.128
15–25	1.405 (0.592–3.335)	0.441
25+[c]		
p53 staining		0.764
0	0.836 (0.403–1.735)	0.630
1	0.650 (0.280–1.510)	0.316
2	0.683 (0.253–1.841)	0.451
3[c]		
Rb staining		0.695
0	0.326 (0.79–1.347)	0.122
1	0.769 (0.253–2.341)	0.644
2	0.687 (0.222–2.131)	0.516
3	0.578 (0.103–3.228)	0.532
4	0.577 (0.164–2.033)	0.392
5[c]		
p16 staining		0.184[b]
0	0.667 (0.367–1.212)	0.184
1[c]		
bcl-2 staining		0.279
0	0.483 (0.190–1.228)	0.126
1	0.646 (0.225–1.852)	0.416
2[c]		

[a] CI, confidence interval.
[b] Reference category.
[c] *P* of variable chosen for the multivariate Cox regression analysis (significant at the 0.25 level).

Table 3 *Multivariate Cox regression analysis of the factors associated with survival*

Step	Variable	Relative risk (95% CI)[a]	*P* (log-rank)
4	Age (yrs)		0.051
	0–29	0.233 (0.062–0.878)	0.031
	30–39	0.273 (0.092–0.812)	0.020
	40–59	0.826 (0.223–3.306)	0.826
	60+[b]		
	Tumor histology		0.347[c]
	Low-grade astrocytoma	1.792 (0.144–22.254)	0.650
	Anaplastic astrocytoma	0.502 (0.143–1.768)	0.284
	Glioblastoma[b]		
	Level of COX-2 expression		0.021
	0–50%	0.275 (0.092–0.821)	0.021
	50–100%[b]		
5	Age (yrs)		0.074[c]
	0–29	0.227 (0.068–0.758)	0.016
	30–39	0.342 (0.130–0.903)	0.030
	40–59	0.533 (0.191–1.491)	0.231
	60+[b]		
	Level of COX-2 expression		0.001
	0–50%	0.208 (0.084–0.516)	0.001
	50–100%[b]		
6	Level of COX-2 expression		<0.001
	0–50%	0.185 (0.076–0.447)	<0.001
	50–100%[b]		

[a] CI, confidence interval.
[b] Reference category.
[c] *P* of variable removed from the stepwise multivariate Cox regression (significant at the 0.05 level).

in the nuclei of tumor cells in a subset of specimens, an observation that to our knowledge has not been reported previously. Its significance is unclear, but this observation may represent the known distribution of COX-2 within the nuclear membrane (66). As has been reported by others, we observed COX-2 staining within neurons, endothelial cells, and reactive astrocytes. The location within reactive astrocytes is consistent with the inducibility of COX-2, and its location within endothelial cells, particularly hyperplastic endothelial cells, supports the concept of PGs having a role in angiogenesis.

We found that the proportion of tumors with high COX-2 expression was greatest in glioblastomas (71%) and lowest in low-grade astrocytomas (30%). The positive association between extent of COX-2 staining and histological grade of gliomas is consistent with the observations of Joki *et al.* (50), who analyzed 50 glioma specimens (25 GBMs, 10 anaplastic astrocytomas, and 15 low-grade astrocytomas) by immunohistochemistry. They reported that 24 of 25 GBMs (96%) had more than 50% COX-2-positive cells, whereas 6 of 10 anaplastic astrocytomas (60%) and only 1 of 15 low-grade astrocytomas (7%) expressed this level of staining. Although some investigators have not found a correlation between COX-2 staining and tumor grade in other tumor types (67), a positive correlation between COX-2 staining and tumor stage has been reported in several human cancers (15, 16, 21). Okami *et al.* (15) demonstrated high COX-2 expression in pancreatic carcinomas but weak staining for COX-2 in benign cystadenomas. Yoshimura *et al.* (16) found marked expression of COX-2 in prostate carcinomas but weak expression of COX-2 in benign prostatic hypertrophy.

In this study, we found that high COX-2 expression was associated with a statistically significant decrease in survival. In multivariate analysis, COX-2 expression predicted outcome independent of all other variables. In fact, COX-2 expression was the strongest predictor of a poor outcome based on Cox regression analyses. In addition, when GBMs were analyzed separately from other tumor grades, increased COX-2 expression remained an important prognostic variable. To our knowledge, a correlation between COX-2 expression and survival of patients with gliomas has not been reported previously. However, increased COX-2 expression has been shown to be a negative prognostic factor in other cancers. Tomozawa *et al.* (68) analyzed 63 patients with colorectal cancer and found that the disease-free survival time for patients with high COX-2 expression was significantly shorter than that for patients with low COX-2 expression. Moreover, among eight prognostic variables (including age, histological type, and Dukes' stage), the only independent significant factor related to disease-free survival in these patients was COX-2 expression. Similarly, Murata *et al.* (69) demonstrated that COX-2 expression potentiated the lymphatic invasion and metastasis of gastric carcinomas. For lung cancer, Achiwa *et al.* (27) demonstrated in a cohort of patients with resected stage I adenocarcinoma that the 5-year survival rate for patients with increased COX-2 expression (66%) was significantly shorter than that for patients with low COX-2 expression (88%). Hida *et al.* (21) reported that the proportion of lung adenocarcinoma cells with marked COX-2 expression was significantly greater in lymph node metastases than in the primary tumor.

We and others have previously demonstrated positive immunostaining for p53 protein in human gliomas (70, 71). Positive staining represents altered p53 protein, which has a longer half-life than wild-type p53. In many but not all tumor specimens with increased p53 immunostaining, a mutation in the *p53* gene is identified (70). Increased p53 immunostaining has been shown to occur with almost equal frequency in all grades of gliomas, although p53 mutations as determined by gene sequencing are more typically found in higher-grade tumors (70, 72). A recent study has suggested that wild-type p53, but not mutant p53, is capable of inhibiting COX-2 expression (38). Wild-type p53 was shown to suppress COX-2 promoter activity by competing with TATA-binding proteins (38). On the basis of this observation, tumor specimens with high p53 immunostaining (indicative of mutant p53 status) should also express high COX-2 levels. However, in our analysis, no statistically significant correlation was detected between high COX-2 expression and p53 immunostaining. Although a trend was observed toward high p53 immunostaining being associated with high COX-2 staining, our data suggest that these two molecular markers are statistically independent of each other. Therefore, although regulation of COX-2 by p53 may be evident *in vitro*, where levels of p53 may be artificially elevated, regulation of COX-2 *in situ* probably depends on multiple variables (14) and thus accounts for the lack of a correlation seen between p53 and COX-2 in our specimens. Similarly, whereas others have demonstrated interactions between COX-2 and bcl-2, a critical antiapoptotic protein that is often overexpressed in gliomas (59–61), we did not find any statistical correlation between bcl-2 and COX-2 expression in our cohort of patients, although tumors with >50% COX-2 staining tended to stain positively for bcl-2. Again, these statistical findings do not nullify the biological possibility that bcl-2 could be one of several factors that can interact with COX-2 expression. For example, epidermal growth factor is also known to up-regulate COX-2 (73, 74), and increased numbers of epidermal growth factor receptors are common in GBMs (48). We also found that the presence of immunostaining for p16 may have been associated with increased COX-2 expression ($P < 0.054$). To date, no interaction between p16 and COX-2 has been described, and the significance of this preliminary finding remains uncertain. In all, it is probable that multiple factors could drive COX-2 expression in gliomas. Additional studies are needed to decipher the interactions between oncogenes, tumor suppressor genes, and COX-2 expression in gliomas.

Although the role of COX-2 in human glioma formation and progression has not been elucidated, it may represent an important therapeutic target. Increased COX-2 expression in rat intestinal epithelial cells has been shown to reduce the apoptotic tendency of these cells (39). Moreover, forced expression of exogenous COX-2 in colon cancer cells increased their metastatic potential and invasiveness (75). Tsujii *et al.* (40) recently demonstrated that COX-2 stimulates colon

cancer cells to release proangiogenic PGs that promote endothelial cell migration and tube formation, which are the initial steps in angiogenesis. Although each of these mechanisms has not been studied specifically in gliomas, Joki *et al.* (50) recently demonstrated that NS-398, a COX-2-specific inhibitor, increased apoptosis, reduced proliferation, and attenuated invasion of cultured human glioma cells. Consequently, COX-2, through PGs and other actions, may influence many of the aggressive features of gliomas. These biological functions of COX-2, along with our finding that increased COX-2 is a significant negative predictor of survival, suggest that COX-2 may be a therapeutic target for treating gliomas. In light of studies in colon cancer that have demonstrated a chemopreventive role for COX-2 inhibition, and because COX-2 expression may drive some of the features of higher grade tumors such as angiogenesis, COX-2 inhibitors may represent novel drugs for preventing the progression of low-grade astrocytomas to anaplastic astrocytomas or GBMs, and in this regard, they may prove to be useful chemopreventive agents. In addition, we recently demonstrated both *in vitro* and in animal models that selective COX-2 inhibitors can sensitize human gliomas to ionizing radiation (76). These observations, taken with the correlations demonstrated in this study between tumor grade and patient survival, support further investigations into the clinical usefulness of COX-2 inhibitors in the treatment of malignant gliomas.

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REFERENCES

1. Taketo, M. M. Cyclooxygenase-2 inhibitors in tumorigenesis (part I). J. Natl. Cancer Inst. (Bethesda), *90:* 1529–1536, 1998.
2. DuBois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S., Van De Putte, L. B., and Lipsky, P. E. Cyclooxygenase in biology and disease. FASEB J., *12:* 1063–1073, 1998.
3. Vane, J. R. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat. New Biol., *231:* 232–235, 1971.
4. Pairet, M., and Engelhardt, G. Distinct isoforms (COX-1 and COX-2) of cyclooxygenase: possible physiological and therapeutic implications. Fundam. Clin. Pharmacol., *10:* 1–17, 1996.
5. Kujubu, D. A., Fletcher, B. S., Varnum, B. C., Lim, R. W., and Herschman, H. R. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J. Biol. Chem., *266:* 12866–12872, 1991.
6. DuBois, R. N., Awad, J., Morrow, J., Roberts, L. J., II, and Bishop, P. R. Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-α and phorbol ester. J. Clin. Investig., *93:* 493–498, 1994.
7. Taketo, M. M. Cyclooxygenase-2 inhibitors in tumorigenesis (part II). J. Natl. Cancer Inst. (Bethesda), *90:* 1609–1620, 1998.
8. Uefuji, K., Ichikura, T., and Mochizuki, H. Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. Clin. Cancer Res., *6:* 135–138, 2000.
9. Higashi, Y., Kanekura, T., and Kanzaki, T. Enhanced expression of cyclooxygenase (COX)-2 in human skin epidermal cancer cells: evidence for growth suppression by inhibiting COX-2 expression. Int. J. Cancer, *86:* 667–671, 2000.
10. Zimmermann, K. C., Sarbia, M., Weber, A. A., Borchard, F., Gabbert, H. E., and Schror, K. Cyclooxygenase-2 expression in human esophageal carcinoma. Cancer Res., *59:* 198–204, 1999.
11. Castelli, M. G., Chiabrando, C., Fanelli, R., Martelli, L., Butti, G., Gaetani, P., and Paoletti, P. Prostaglandin and thromboxane synthesis by human intracranial tumors. Cancer Res., *49:* 1505–1508, 1989.
12. Molina, M. A., Sitja-Arnau, M., Lemoine, M. G., Frazier, M. L., and Sinicrope, F. A. Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines: growth inhibition by nonsteroidal anti-inflammatory drugs. Cancer Res., *59:* 4356–4362, 1999.
13. Wardlaw, S. A., March, T. H., and Belinsky, S. A. Cyclooxygenase-2 expression is abundant in alveolar type II cells in lung cancer-sensitive mouse strains and in premalignant lesions. Carcinogenesis (Lond.), *21:* 1371–1377, 2000.
14. Yip-Schneider, M. T., Barnard, D. S., Billings, S. D., Cheng, L., Heilman, D. K., Lin, A., Marshall, S. J., Crowell, P. L., Marshall, M. S., and Sweeney, C. J. Cyclooxygenase-2 expression in human pancreatic adenocarcinomas. Carcinogenesis (Lond.), *21:* 139–146, 2000.
15. Okami, J., Yamamoto, H., Fujiwara, Y., Tsujie, M., Kondo, M., Noura, S., Oshima, S., Nagano, H., Dono, K., Umeshita, K., Ishikawa, O., Sakon, M., Matsuura, N., Nakamori, S., and Monden, M. Overexpression of cyclooxygenase-2 in carcinoma of the pancreas. Clin. Cancer Res., *5:* 2018–2024, 1999.
16. Yoshimura, R., Sano, H., Masuda, C., Kawamura, M., Tsubouchi, Y., Chargui, J., Yoshimura, N., Hla, T., and Wada, S. Expression of cyclooxygenase-2 in prostate carcinoma. Cancer (Phila.), *89:* 589–596, 2000.
17. Kargman, S. L., O'Neill, G. P., Vickers, P. J., Evans, J. F., Mancini, J. A., and Jothy, S. Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. Cancer Res., *55:* 2556–2559, 1995.
18. Kutchera, W., Jones, D. A., Matsunami, N., Groden, J., McIntyre, T. M., Zimmerman, G. A., White, R. L., and Prescott, S. M. Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: evidence for a transcriptional effect. Proc. Natl. Acad. Sci. USA, *93:* 4816–4820, 1996.
19. Battu, S., Chable-Rabinovitch, H., Rigaud, M., and Beneytout, J. L. Cyclooxygenase-2 expression in human adenocarcinoma cell line HT29 cl.19A. Anticancer Res., *18:* 2397–2403, 1996.
20. Chan, G., Boyle, J. O., Yang, E. K., Zhang, F., Sacks, P. G., Shah, J. P., Edelstein, D., Soslow, R. A., Koki, A. T., Woerner, B. M., Masferrer, J. L., and Dannenberg, A. J. Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. Cancer Res., *59:* 991–994, 1999.
21. Hida, T., Yatabe, Y., Achiwa, H., Muramatsu, H., Kozaki, K., Nakamura, S., Ogawa, M., Mitsudomi, T., Sugiura, T., and Takahashi, T. Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Cancer Res., *58:* 3761–3764, 1998.
22. Palayoor, S. T., Bump, E. A., Calderwood, S. K., Bartol, S., and Coleman, C. N. Combined antitumor effect of radiation and ibuprofen in human prostate carcinoma cells. Clin. Cancer Res., *4:* 763–771, 1998.
23. Paoletti, P., Chiabrando, C., Gaetani, P., Castelli, M. G., Butti, G., Martelli, L., and Rolli, M. Prostaglandins in human brain tumors. J. Neurosurg. Sci., *33:* 65–69, 1989.
24. Eberhart, C. E., Coffey, R. J., Radhika, A., Giardiello, F. M., Ferrenbach, S., and DuBois, R. N. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology, *107:* 1183–1188, 1994.
25. Sano, H., Kawahito, Y., Wilder, R. L., Hashiramoto, A., Mukai, S., Asai, K., Kimura, S., Kato, H., Kondo, M., and Hla, T. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res., *55:* 3785–3789, 1995.
26. Subbaramaiah, K., Telang, N., Ramonetti, J. T., Araki, R., DeVito, B., Weksler, B. B., and Dannenberg, A. J. Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. Cancer Res., *56:* 4424–4429, 1996.
27. Achiwa, H., Yatabe, Y., Hida, T., Kuroishi, T., Kozaki, K., Nakamura, S., Ogawa, M., Sugiura, T., Mitsudomi, T., and Takahashi, T. Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinomas. Clin. Cancer Res., *5:* 1001–1005, 1999.
28. Kondo, M., Yamamoto, H., Nagano, H., Okami, J., Ito, Y., Shimizu, J., Eguchi, H., Miyamoto, A., Dono, K., Umeshita, K., Matsuura, N., Wakasa, K., Nakamori, S., Sakon, M., and Monden, M. Increased expression of COX-2 in nontumor liver tissue is associated with shorter disease-free survival in patients with hepatocellular carcinoma. Clin. Cancer Res., *5:* 4005–4012, 1999.
29. Sheehan, K. M., Sheahan, K., O'Donoghue, D. P., MacSweeney, F., Conroy, R. M., Fitzgerald, D. J., and Murray, F. E. The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA, *282:* 1254–1257, 1999.
30. Giardiello, F. M., Hamilton, S. R., Krush, A. J., Piantadosi, S., Hylind, L. M., Celano, P., Booker, S. V., Robinson, C. R., and Offerhaus, G. J. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N. Engl. J. Med., *328:* 1313–1316, 1993.
31. Kune, G. A., Kune, S., and Watson, L. F. Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. Cancer Res., *48:* 4399–4404, 1988.
32. Thun, M. J., Namboodiri, M. M., and Heath, C. W., Jr. Aspirin use and reduced risk of fatal colon cancer. N. Engl. J. Med., *325:* 1593–1596, 1991.
33. Jacoby, R. F., Seibert, K., Cole, C. E., Kelloff, G., and Lubet, R. A. The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. Cancer Res., *60:* 5040–5044, 2000.
34. Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J. M., Evans, J. F., and Taketo, M. M. Suppression of intestinal polyposis in Apc Δ716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell, *87:* 803–809, 1996.
35. Reddy, B. S., Rao, C. V., and Seibert, K. Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. Cancer Res., *56:* 4566–4569, 1996.
36. Rao, C. V., Rivenson, A., Simi, B., Zang, E., Kelloff, G., Steele, V., and Reddy, B. S. Chemoprevention of colon carcinogenesis by sulindac, a nonsteroidal anti-inflammatory agent. Cancer Res., *55:* 1464–1472, 1995.
37. Piazza, G. A., Rahm, A. K., Finn, T. S., Fryer, B. H., Li, H., Stoumen, A. L., Pamukcu, R., and Ahnen, D. J. Apoptosis primarily accounts for the growth-inhibitory properties of sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest, and p53 induction. Cancer Res., *57:* 2452–2459, 1997.
38. Subbaramaiah, K., Altorki, N., Chung, W. J., Mestre, J. R., Sampat, A., and Dannenberg, A. J. Inhibition of cyclooxygenase-2 gene expression by p53. J. Biol. Chem., *274:* 10911–10915, 1999.
39. Tsujii, M., and DuBois, R. N. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell, *83:* 493–501, 1995.
40. Tsujii, M., Kawano, S., Tsuji, S., Sawaoka, H., Hori, M., and DuBois, R. N. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell, *93:* 705–716, 1998.

41. Liu, X. H., Kirschenbaum, A., Yao, S., Stearns, M. E., Holland, J. F., Claffey, K., and Levine, A. C. Upregulation of vascular endothelial growth factor by cobalt chloride-simulated hypoxia is mediated by persistent induction of cyclooxygenase-2 in a metastatic human prostate cancer cell line. Clin. Exp. Metastasis, *17:* 687–694, 1999.
42. Liu, X. H., Kirschenbaum, A., Yao, S., Lee, R., Holland, J. F., and Levine, A. C. Inhibition of cyclooxygenase-2 suppresses angiogenesis and the growth of prostate cancer *in vivo*. J. Urol., *164:* 820–825, 2000.
43. Attiga, F. A., Fernandez, P. M., Weeraratna, A. T., Manyak, M. J., and Patierno, S. R. Inhibitors of prostaglandin synthesis inhibit human prostate tumor cell invasiveness and reduce the release of matrix metalloproteinases. Cancer Res., *60:* 4629–4637, 2000.
44. Aggarwal, S., Taneja, N., Lin, L., Orringer, M. B., Rehemtulla, A., and Beer, D. G. Indomethacin-induced apoptosis in esophageal adenocarcinoma cells involves up-regulation of bax and translocation of mitochondrial cytochrome *c* independent of COX-2 expression. Neoplasia, *2:* 346–356, 2000.
45. Bissonnette, M., Khare, S., von Lintig, F. C., Wali, R. K., Nguyen, L., Zhang, Y., Hart, J., Skarosi, S., Varki, N., Boss, G. R., and Brasitus, T. A. Mutational and nonmutational activation of p21ras in rat colonic azoxymethane-induced tumors: effects on mitogen-activated protein kinase, cyclooxygenase-2, and cyclin D1. Cancer Res., *60:* 4602–4609, 2000.
46. Shrieve, D. C., Alexander, E., III, Black, P. M., Wen, P. Y., Fine, H. A., Kooy, H. M., and Loeffler, J. S. Treatment of patients with primary glioblastoma multiforme with standard postoperative radiotherapy and radiosurgical boost: prognostic factors and long-term outcome. J. Neurosurg., *90:* 72–77, 1999.
47. Kleihues, P., and Cavenee, W. K. Pathology and Genetics of Tumours of the Nervous System. Lyon, France: IARC Press, 2000.
48. Lang, F. F., Miller, D. C., Koslow, M., and Newcomb, E. W. Pathways leading to glioblastoma multiforme: a molecular analysis of genetic alterations in 65 astrocytic tumors. J. Neurosurg., *81:* 427–436, 1994.
49. Deininger, M. H., Weller, M., Streffer, J., Mittelbronn, M., and Meyermann, R. Patterns of cyclooxygenase-1 and -2 expression in human gliomas *in vivo*. Acta Neuropathol. (Berl.), *98:* 240–244, 1999.
50. Joki, T., Heese, O., Nikas, D. C., Bello, L., Zhang, J., Kraeft, S. K., Seyfried, N. T., Abe, T., Chen, L. B., Carroll, R. S., and Black, P. M. Expression of cyclooxygenase 2 (COX-2) in human glioma and *in vitro* inhibition by a specific COX-2 inhibitor, NS-398. Cancer Res., *60:* 4926–4931, 2000.
51. Kyritsis, A. P., Bondy, M. L., Hess, K. R., Cunningham, J. E., Zhu, D., Amos, C. J., Yung, W. K., Levin, V. A., and Bruner, J. M. Prognostic significance of p53 immunoreactivity in patients with glioma. Clin. Cancer Res., *1:* 1617–1622, 1995.
52. Kyritsis, A. P., Xu, R., Bondy, M. L., Levin, V. A., and Bruner, J. M. Correlation of p53 immunoreactivity and sequencing in patients with glioma. Mol. Carcinog., *15:* 1–4, 1996.
53. Kyritsis, A. P., Zhang, B., Zhang, W., Xiao, M., Takeshima, H., Bondy, M. L., Cunningham, J. E., Levin, V. A., and Bruner, J. Mutations of the *p16* gene in gliomas. Oncogene, *12:* 63–67, 1996.
54. Fueyo, J., Gomez-Manzano, C., Bruner, J. M., Saito, Y., Zhang, B., Zhang, W., Levin, V. A., Yung, W. K., and Kyritsis, A. P. Hypermethylation of the CpG island of p16/CDKN2 correlates with gene inactivation in gliomas. Oncogene, *13:* 1615–1619, 1996.
55. Puduvalli, V. K., Kyritsis, A. P., Hess, K. R., Bondy, M. L., Fuller, G. N., Levin, V. A., and Bruner, J. M. Patterns of expression of Rb and p16 in gliomas and correlation with survival. Int. J. Oncol., *17:* 963–969, 2000.
56. Heck, K., Pyle, P., and Bruner, J. M. Bcl-2 expression in cerebellar medulloblastomas. Brain Pathol., *4:* 430A, 1994.
57. Bruner, J. M., Connelly, J. H., and Saya, H. p53 protein immunostaining in routinely processed paraffin-embedded sections. Mod. Pathol., *6:* 189–194, 1993.
58. Pollack, I. F., Campbell, J. W., Hamilton, R. L., Martinez, A. J., and Bozik, M. E. Proliferation index as a predictor of prognosis in malignant gliomas of childhood. Cancer (Phila.), *79:* 849–856, 1997.
59. Krishna, M., Smith, T. W., and Recht, L. D. Expression of bcl-2 in reactive and neoplastic astrocytes: lack of correlation with presence or degree of malignancy. J. Neurosurg., *83:* 1017–1022, 1995.
60. Nakasu, S., Nakasu, Y., Nioka, H., Nakajima, M., and Handa, J. bcl-2 protein expression in tumors of the central nervous system. Acta Neuropathol., *88:* 520–526, 1994.
61. Alderson, L. M., Castleberg, R. L., Harsh, G. R. T., Louis, D. N., and Henson, J. W. Human gliomas with wild-type p53 express bcl-2. Cancer Res., *55:* 999–1001, 1995.
62. Sairanen, T., Ristimaki, A., Karjalainen-Lindsberg, M. L., Paetau, A., Kaste, M., and Lindsberg, P. J. Cyclooxygenase-2 is induced globally in infarcted human brain. Ann. Neurol., *43:* 738–747, 1998.
63. Miettinen, S., Fusco, F. R., Yrjanheikki, J., Keinanen, R., Hirvonen, T., Roivainen, R., Narhi, M., Hokfelt, T., and Koistinaho, J. Spreading depression and focal brain ischemia induce cyclooxygenase-2 in cortical neurons through *N*-methyl-D-aspartic acid-receptors and phospholipase A2. Proc. Natl. Acad. Sci. USA, *94:* 6500–6505, 1997.
64. Elder, D. J., Halton, D. E., Hague, A., and Paraskeva, C. Induction of apoptotic cell death in human colorectal carcinoma cell lines by a cyclooxygenase-2 (COX-2)-selective nonsteroidal anti-inflammatory drug: independence from COX-2 protein expression. Clin. Cancer Res., *3:* 1679–1683, 1997.
65. Tsuji, S., Kawano, S., Sawaoka, H., Takei, Y., Kobayashi, I., Nagano, K., Fusamoto, H., and Kamada, T. Evidence for involvement of cyclooxygenase-2 in proliferation of two gastrointestinal cancer cell lines. Prostaglandins Leukotrienes Essent. Fatty Acids, *55:* 179–183, 1996.
66. Morita, I., Schindler, M., Regier, M. K., Otto, J. C., Hori, T., DeWitt, D. L., and Smith, W. L. Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. J. Biol. Chem., *270:* 10902–10908, 1995.
67. Sung, J. J., Leung, W. K., Go, M. Y., To, K. F., Cheng, A. S., Ng, E. K., and Chan, F. K. Cyclooxygenase-2 expression in *Helicobacter pylori*-associated premalignant and malignant gastric lesions. Am. J. Pathol., *157:* 729–735, 2000.
68. Tomozawa, S., Tsuno, N. H., Sunami, E., Hatano, K., Kitayama, J., Osada, T., Saito, S., Tsuruo, T., Shibata, Y., and Nagawa, H. Cyclooxygenase-2 overexpression correlates with tumour recurrence, especially haematogenous metastasis, of colorectal cancer. Br. J. Cancer, *83:* 324–328, 2000.
69. Murata, H., Kawano, S., Tsuji, S., Tsuji, M., Sawaoka, H., Kimura, Y., Shiozaki, H., and Hori, M. Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric carcinoma. Am. J. Gastroenterol., *94:* 451–455, 1999.
70. Lang, F. F., Miller, D. C., Pisharody, S., Koslow, M., and Newcomb, E. W. High frequency of p53 protein accumulation without *p53* gene mutation in human juvenile pilocytic, low grade and anaplastic astrocytomas. Oncogene, *9:* 949–954, 1994.
71. von Deimling, A., Eibl, R. H., Ohgaki, H., Louis, D. N., von Ammon, K., Petersen, I., Kleihues, P., Chung, R. Y., Wiestler, O. D., and Seizinger, B. R. p53 mutations are associated with 17p allelic loss in grade II and grade III astrocytoma. Cancer Res., *52:* 2987–2990, 1992.
72. Sidransky, D., Mikkelsen, T., Schwechheimer, K., Rosenblum, M. L., Cavanee, W., and Vogelstein, B. Clonal expansion of p53 mutant cells is associated with brain tumour progression. Nature (Lond.), *355:* 846–847, 1992.
73. Mestre, J. R., Subbaramaiah, K., Sacks, P. G., Schantz, S. P., Tanabe, T., Inoue, H., and Dannenberg, A. J. Retinoids suppress epidermal growth factor-induced transcription of cyclooxygenase-2 in human oral squamous carcinoma cells. Cancer Res., *57:* 2890–2895, 1997.
74. Sato, T., Nakajima, H., Fujio, K., and Mori, Y. Enhancement of prostaglandin E2 production by epidermal growth factor requires the coordinate activation of cytosolic phospholipase A2 and cyclooxygenase 2 in human squamous carcinoma A431 cells. Prostaglandins, *53:* 355–369, 1997.
75. Tsujii, M., Kawano, S., and DuBois, R. N. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc. Natl. Acad. Sci. USA, *94:* 3336–3340, 1997.
76. Petersen, C., Petersen, S., Milas, L., Lang, F. F., and Tofilon, P. J. Enhancement of intrinsic tumor cell radiosensitivity induced by a selective cyclooxygenase-2 inhibitor. Clin. Cancer Res., *6:* 2513–2520, 2000.