Cyclooxygenase-2 Expression Is an Independent Predictor of Poor Prognosis in Colon Cancer

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Abstract

Purpose: Cyclooxygenase-2 (COX-2; *PTGS2*) is considered to play an important role in colorectal carcinogenesis and is often up-regulated in colon cancers. However, previous data on the influence of COX-2 expression on patient outcome have been conflicting.

Experimental Design: Using 662 colon cancers (stage I-IV) in two independent prospective cohorts (the Nurses' Health Study and the Health Professionals Follow-up Study), we detected COX-2 overexpression in 548 (83%) tumors by immunohistochemistry. Cox proportional hazards models were used to compute hazard ratios (HR) of colon cancer-specific and overall mortalities, adjusted for patient characteristics and related molecular events, including the CpG island methylation phenotype, microsatellite instability, and p53, CIMP, *KRAS*, and *BRAF* mutations.

Results: During follow-up of the 662 cases, there were 283 deaths, including 163 colon cancer-specific deaths. Patients with COX-2-positive tumors showed a trend towards an inferior colon cancer-specific mortality [HR, 1.37; 95% confidence interval (95% CI), 0.87-2.14], which became significant after adjusting for tumor stage and other predictors of clinical outcome (multivariate HR, 1.70; 95% CI, 1.06-2.74; $P = 0.029$). Notably, the prognostic effect of COX-2 expression might differ according to p53 status ($P_{interaction} = 0.04$). Compared with tumors with both COX-2 and p53 negative, COX-2-positive tumors were significantly associated with an increased cancer-specific mortality (multivariate HR, 2.12; 95% CI, 1.23-3.65) regardless of p53 status. A similar trend was observed when overall mortality was used as an outcome.

Conclusion: COX-2 overexpression is associated with worse survival among colon cancer patients. The effect of COX-2 on clinical outcome may be modified by p53 status.

Cyclooxygenase-2 (COX-2; *PTGS2*) converts arachidonic acid to prostaglandins and related eicosanoids and promotes inflammation and cell proliferation (1, 2). COX-2 is overexpressed in the majority of human colon cancers (2–4). Supporting the importance of COX-2 in colorectal carcinogenesis, randomized trials have shown that aspirin and COX-2 selective inhibitors reduce risk of recurrent adenoma among high-risk patients (5–7).

Despite the well-accepted role of COX-2 in tumor development (2), studies are conflicting regarding prognostic significance of COX-2 in colorectal cancer with some (3, 8, 9) supporting and others (4, 10–16) refuting an independent adverse effect of COX-2 overexpression. COX-2 overexpression has been positively associated with p53 alteration (17, 18) and inversely associated with microsatellite instability (MSI; refs. 18–20), which generally predicts longer survival of colon cancer patients (21). Moreover, COX-2 and p53 appear to regulate each other in a complex manner (17, 22, 23). Thus, effect of COX-2 on patient survival can possibly be confounded by p53 alteration, MSI, and other related molecular events.

In this study using a large number ($n = 662$) of colon cancer patients in two independent cohort studies, we have examined the effect of tumoral COX-2 expression on patient outcome adjusted for tumor stage and other potential predictors of clinical outcome. Because we concurrently assessed tumoral molecular alterations including p53, *KRAS* and *BRAF* mutations, MSI, and the CpG island methylator phenotype (CIMP), we could evaluate the independent effect of COX-2 expression after controlling for these related molecular events.

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Translational Relevance

COX-2 has been shown to play an important role in carcinogenesis in various organ systems including colon. COX-2 inhibitors (aspirin, nonsteroidal anti-inflammatory drugs, and celecoxib) have been shown to be effective in preventing colorectal adenoma and cancer. However, the relation between COX-2 expression in colon cancer and patient survival has been controversial. We have used the database of ›600 colon cancer in two independent, prospective cohort studies, with available clinical information, adequate follow-up, and other important molecular events in colon cancers. To our knowledge, this is the first study to show adverse effect of COX-2 overexpression on clinical outcome independent of related molecular events including *BRAF* mutation, MSI, and CIMP, all of which are associated with both COX-2 expression and clinical outcome in colon cancer. Thus, our findings are relevant to practice in oncology.

Materials and Methods

Study population. We used the databases of two large prospective cohort studies; the Nurses' Health Study (n = 121,700 women followed since 1976; refs. 24, 25) and the Health Professionals' Follow-up Study (n = 51,500 men followed since 1986; ref. 25). On each biennial follow-up questionnaire, participants were asked whether they had a diagnosis of colon cancer during the previous 2 years. When a participant (or next of kin for decedents) reported colon cancer, we sought permission to obtain medical records. Study physicians, while blinded to exposure data, reviewed all records related to colon cancer and recorded American Joint Committee on Cancer tumor stage and tumor location. For nonresponders, we searched the National Death Index to discover deaths and ascertain any diagnosis of colon cancer that contributed to death or was a secondary diagnosis. Approximately 96% of all incident colon cancer cases were identified through these methods. We collected paraffin-embedded tissue blocks from hospitals where colon cancer patients underwent resections of primary tumors (25). Tissue sections from all colon cancer cases were reviewed and confirmed by a pathologist (S.O.). Tumor grade was categorized as high (≤50% glandular area) or low (>50% glandular area). Based on availability of tissue samples, we included a total of 662 colon cancer cases (287 from the men's cohort and 375 from the women's cohort) diagnosed up to 2002. Written informed consent was obtained from all subjects. This study was approved by the human subjects committees at Brigham and Women's Hospital and the Harvard School of Public Health.

Measurement of mortality. Patients were observed until death or June 2006, whichever came first. Ascertainment of deaths included reporting by the family or postal authorities. In addition, the names of persistent nonresponders were searched in the National Death Index. The cause of death was assigned by physicians blinded to other clinical and lifestyle information. More than 98% of deaths in the cohorts were identified by these methods.

Immunohistochemistry for COX-2 and p53. Tissue microarrays construction and immunohistochemical examination for COX-2 and p53 were done as described previously (18). p53 positivity was defined as ≥50% of tumor cells with unequivocal strong nuclear staining. These criteria were based on the observations that the 50% cutoff appeared to increase specificity of p53 immunohistochemistry to correlate with the presence of *TP53* mutation (26–28). Our data also indicated that MSI-high or CIMP-high was uncommon (6.3-8.1%) in tumors with p53 positivity in ≥50% tumor cells, whereas CIMP-high or MSI-high was more frequent (22-27%) in tumors with p53 positivity in <50% tumor cells as well as tumors without p53 staining.

For COX-2 immunohistochemistry, antigen retrieval was done by incubating deparaffinized tissue sections in citrate buffer (BioGenex) by a microwave for 15 min and let the sections cool for at least 40 min. Tissue sections were incubated with 3% H_2O_2 (20 min) to block endogenous peroxidase and then incubated with avidin block (Vector Laboratories; 15 min) then with biotin block (Vector Laboratories; 15 min). Primary anti-COX-2 antibody (Cayman Chemical; dilution 1:300) was applied overnight at 4°C. Then, secondary anti-mouse antibody (Vector Laboratories) was applied (20 min), avidin-biotin

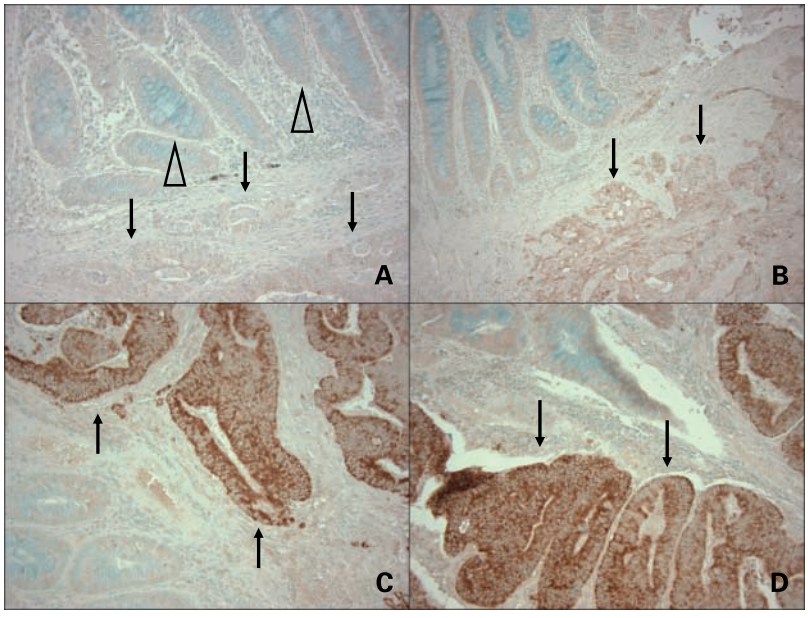

Fig. 1. COX-2 expression in colon cancer. *A,* no COX-2 overexpression in colon cancer (*arrow*) or normal colonic mucosa (*empty arrowheads*). *B,* weak COX-2 overexpression in colon cancer (*arrows*). *C* and *D,* strong COX-2 overexpression in colon cancer (*arrows*).

complex conjugate (Vector Laboratories) was added, and sections were visualized by diaminobenzidine (5 min) and methyl green counterstain. For each assay run, we included a positive control (cancer with COX-2 overexpression) and a negative control (normal colonic tissue). We also treated a positive control specimen with PBS without anti-COX-2 antibody. A pathologist (S.O.), unaware of other data, interpreted cytoplasmic COX-2 expression in tumor as either absent, weak, moderate, or strong staining compared with adjacent normal colonic epithelium. Inflammatory cells served as internal built-in positive controls (18, 25). Consistent with other investigators (8, 29), if immunostaining intensity was moderate or strong, tumors were classified as cancers with COX-2 overexpression. If immunostaining intensity was weak or absent, tumors were classified as cancers with negative COX-2 overexpression (Fig. 1). This classification has been shown previously to associate well with p53 expression and inversely with MSI and CIMP in colorectal cancer (18).

Appropriate positive and negative controls were included in each run of immunohistochemistry. All immunohistochemically stained slides were interpreted by a pathologist (S.O.) unaware of other data. A random sample of 108 cases was reexamined for COX-2 expression by a second observer (R.D.) unaware of other data, and the concordance between the two observers was 0.92 ($\kappa = 0.62$; $P < 0.0001$), indicating substantial agreement. Another random sample of 118 tumors was reexamined for p53 by another observer (K.N.), unaware of other data, and the concordance between the two observers was 0.87 ($\kappa = 0.75$; $P < 0.0001$).

Genomic DNA extraction and sequencing of KRAS and BRAF. Genomic DNA from paraffin-embedded tissue and whole genome amplification of genomic DNA was done as described previously (30). PCR and sequencing targeted for *KRAS* codons 12 and 13 and *BRAF* codon 600 were done as described previously (30, 31).

MSI analysis. MSI status was determined using a microsatellite marker panel consisting of D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487 (a 10-marker panel; ref. 32). A high degree of MSI (MSI-high) was defined as the presence of instability in ≥30% of the markers, MSI-low as the presence of instability in <30% of markers, and microsatellite stability as no unstable marker.

Table 1. Clinical and molecular features of colon cancer according to COX-2 expression

Clinical or molecular feature	All cases	COX-2 negative	COX-2 positive	*P*
Total *n*	662	114	548	
Sex				
Male (HPFS)	287 (43)	52 (46)	235 (43)	0.59
Female (NHS)	375 (57)	62 (54)	313 (57)	
Age, mean ± SD	66.5 ± 8.3	66.8 ± 7.4	66.5 ± 8.4	0.69
Year of diagnosis				
Before 1990	101 (15)	17 (15)	84 (15)	0.05
1990-1999	482 (73)	91 (80)	391 (71)	
2000-2002	79 (12)	6 (5.3)	73 (13)	
Tumor location*				
Proximal	434 (59)	80 (71)	305 (56)	0.005
Distal	297 (41)	33 (29)	236 (44)	
Tumor stage				
I	136 (21)	25 (22)	111 (20)	0.77
IIA	207 (31)	40 (35)	167 (30)	
IIB	20 (3.0)	1 (0.9)	19 (3.5)	
IIIA	21 (3.2)	2 (1.8)	19 (3.5)	
IIIB	88 (13)	13 (11)	75 (14)	
IIIC	55 (8.3)	13 (11)	42 (7.7)	
IV	86 (13)	15 (13)	71 (13)	
Unknown	49 (7.4)	5 (4.4)	44 (8.0)	
Tumor grade				
Low	585 (89)	90 (79)	495 (91)	0.0003
High	74 (11)	24 (21)	50 (9.2)	
p53 †				
-	404 (61)	91 (81)	313 (58)	<0.0001
+	253 (39)	22 (19)	231 (42)	
MSI				
MSI-low/MSS	521 (81)	77 (69)	446 (84)	0.0009
MSI-high	121 (19)	33 (31)	88 (16)	
CIMP				
CIMP-0	277 (42)	32 (28)	245 (45)	0.004
CIMP-low	257 (39)	53 (46)	204 (37)	
CIMP-high	128 (19)	29 (25)	99 (18)	
KRAS mutation				
-	411 (64)	68 (61)	343 (64)	0.55
+	234 (36)	43 (39)	191 (36)	
BRAF mutation				
-	525 (83)	85 (79)	440 (84)	0.24
+	105 (17)	22 (21)	83 (16)	

NOTE: Numbers in parentheses indicate the proportion of tumors with a specific clinical or molecular feature in a given COX-2 subtype.
Abbreviations: HPFS, Health Professionals' Follow-up Study; NHS, Nurses' Health Study; MSS, microsatellite stable.
*Proximal colon includes cecum to transverse colon and distal colon includes splenic flexure to sigmoid colon.
† p53 status was determined by immunohistochemistry.

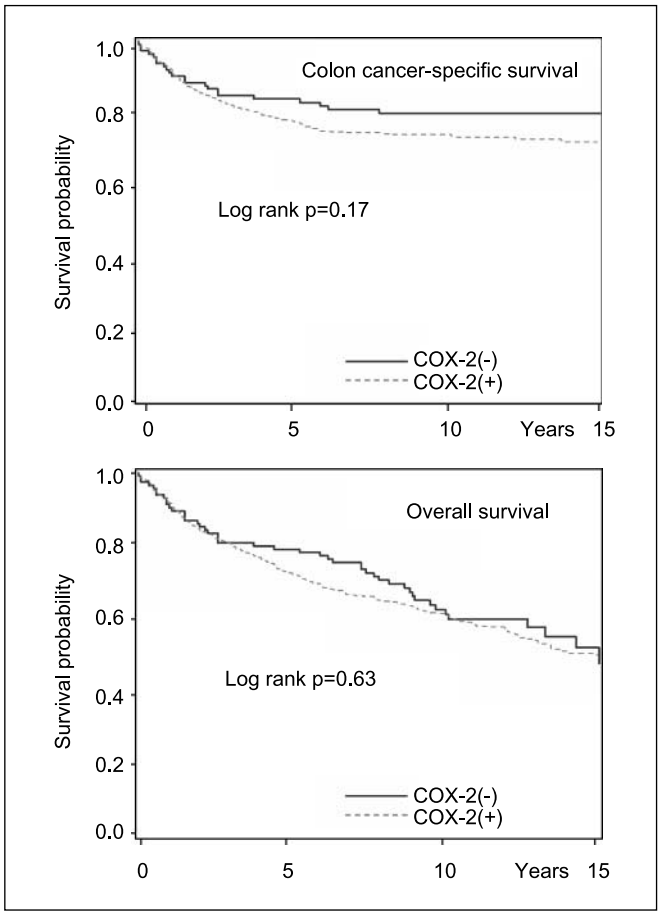

Fig. 2. Kaplan-Meier survival curves in colon cancer according to tumoral COX-2 status.

Real-time PCR (MethyLight) for quantitative DNA methylation analysis. Sodium bisulfite treatment on DNA and MethyLight assays were validated and done as described previously (33). We used ABI 7300 (Applied Biosystems) for quantitative real-time PCR (MethyLight; ref. 34) on 8 CIMP-specific markers [*CACNA1G*, *CDKN2A* (*p16*), *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1*; refs. 32, 35]. CIMP-high was defined as ≥6 of 8 methylated markers using the 8-marker CIMP panel, CIMP-low as 1 to 5 of 8 methylated markers, and CIMP-0 as 0/8 methylated markers according to the previously established criteria (32).

Statistical analysis. We used Cox proportional hazards models to calculate hazard ratios (HR) of death according to tumoral COX-2 status, unadjusted as well as adjusted for age, sex, year of diagnosis, tumor location, stage, grade, MSI, CIMP, *KRAS*, *BRAF*, and p53. For analyses of colon cancer-specific mortality, death as a result of colon cancer was the primary endpoint and deaths as a result of other causes were censored. To adjust for potential confounding, age and year of diagnosis were used as continuous variables, and all of the other covariates were used as categorical variables. We dichotomized tumor location (proximal versus distal), tumor grade (high versus low), CIMP (high versus low/0), MSI (high versus low/microsatellite stable), p53 (positive versus negative), *KRAS* (mutated versus wild-type), and *BRAF* (mutated versus wild-type). We assigned a separate indicator variable to each tumor stage (as in Table 1) to minimize residual confounding. When there was missing information on tumor location (1.2% missing), stage (7.4% missing), tumor grade (0.5% missing), MSI (3.0% missing), p53 (0.8% missing), *KRAS* (2.6% missing), or *BRAF* (4.8% missing), we assigned a separate ("missing") indicator variable and included those cases in the multivariate analysis models. We confirmed that excluding cases with a missing variable did not significantly alter results (data not shown). An interaction was assessed by including the cross-product of the COX-2 variable and another variable of interest in a multivariate Cox model, and the likelihood ratio test was done. To assess an interaction of COX-2 and stage, we dichotomized tumor stage (I-II versus III-IV). The Kaplan-Meier method was used to describe the distribution of colon cancer-specific and overall survival time, and the log-rank test was done. The χ^2 test was used to examine an association between categorical variables. The *t* test assuming unequal variances was done to compare mean age. All analyses used SAS version 9.1 (SAS Institute) and all *P* values were two-sided.

Results

COX-2 expression in colon cancers. Among the 662 tumors, 548 (83%) were positive for overexpression of COX-2, whereas 114 (17%) were negative for COX-2. We also examined p53 status (by immunohistochemistry), MSI, CIMP, and *KRAS* and *BRAF* mutations, because expressions of COX-2 and p53 were inversely related with MSI and CIMP (18), and MSI, CIMP, and *BRAF* mutation have been related with patient outcome (21, 36–39). Table 1 summarizes clinical and molecular features of colon cancer according to COX-2 status. Notably, compared with COX-2-negative tumors, COX-2-positive tumors are more likely distal, low grade, and p53 positive and less likely MSI-high and CIMP-high.

Table 2. COX-2 expression and survival among colon cancer patients

	Total *n* (%)	Colon cancer-specific mortality				Overall mortality			
		Deaths/ person-years	Univariate HR (95% CI)	Stage-adjusted HR (95% CI)	Multivariate HR (95% CI)	Deaths/ person-years	Univariate HR (95% CI)	Stage-adjusted HR (95% CI)	Multivariate HR (95% CI)
COX-2 negative	114 (17)	22/ 1,041	1 (reference)	1 (reference)	1 (reference)	47/ 1,041	1 (reference)	1 (reference)	1 (reference)
COX-2 positive	548 (83)	141/ 4,834	1.37 (0.87-2.14)	1.57 (1.00-2.46)	1.70 (1.06-2.74)	236/ 4,834	1.08 (0.79-1.48)	1.21 (0.88-1.66)	1.21 (0.87-1.69)
P			0.17	0.051	0.029		0.63	0.23	0.26

NOTE: The multivariate Cox model includes age, year of diagnosis, sex, tumor location, stage, grade, and status of *KRAS, BRAF*, p53, MSI, and CIMP.

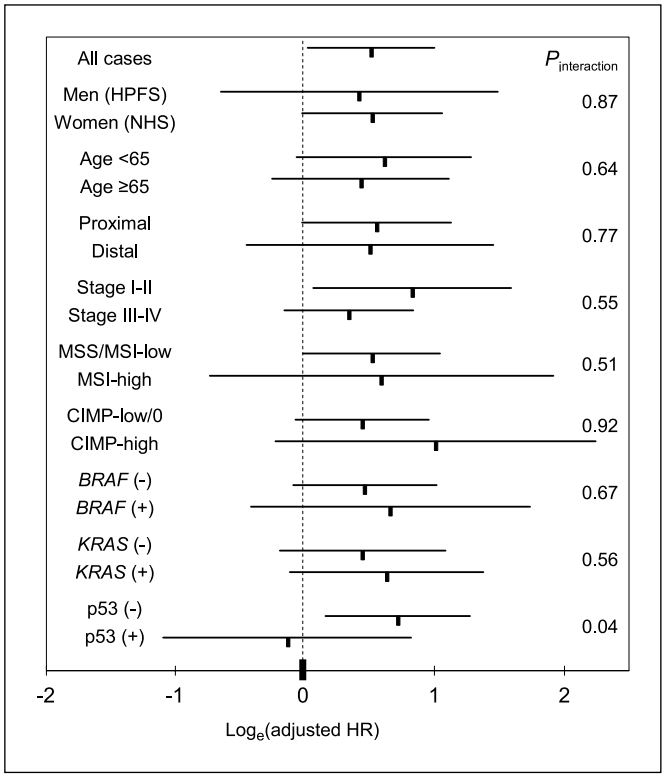

Fig. 3. Stratified analysis of colon cancer-specific mortality in COX-2-positive tumors. Log$_e$ (adjusted HR) with 95% CI for COX-2-positive tumors (versus COX-2-negative tumors) in various strata. HPFS, Health Professionals' Follow-up Study; NHS, Nurses' Health Study.

COX-2 expression and patient survival in colon cancer. Among the 662 eligible patients with adequate follow-up, there were 283 deaths, including 163 colon cancer-specific deaths. We assessed the influence of COX-2 expression on patient survival. Five-year colon cancer-specific survival was 84% among patients with COX-2-negative tumors and 78% among patients with COX-2-positive tumors (log-rank P = 0.17; Fig. 2). Five-year overall survival was 79% among patients with COX-2-negative tumors and 72% among those with COX-2-positive tumors (log-rank P = 0.63).

In univariate Cox regression analysis, COX-2 positivity was associated with a nonsignificant increase in colon cancer-specific mortality [HR, 1.37; 95% confidence interval (95% CI), 0.87-2.14; Table 2]. In a multivariate model that adjusted for other clinical, pathologic, and molecular predictors of survival, COX-2 positivity was associated with a significant increase in colon cancer-specific mortality (multivariate HR, 1.70; 95% CI, 1.06-2.74). The increase in the effect of COX-2 positivity on survival in the multivariate analysis was mainly the result of adjusting for tumor stage; when we simply adjusted for tumor stage, the HR for colon cancer-specific mortality in COX-2-positive tumors was 1.57 (95% CI, 1.00-2.46). When we excluded stage IV cases, multivariate HR for colon cancer-specific mortality in COX-2-positive cases (versus COX-2-negative cases) was 1.60 (95% CI, 0.81-3.13). Thus, the results did not change substantially.

COX-2 expression did not significantly influence overall mortality in both univariate and multivariate analyses (Table 2). High tumor grade was associated with an increased colon cancer-specific mortality (multivariate HR, 2.07; 95% CI, 1.17-3.66). p53 positivity was not a significant predictor of survival in both univariate and multivariate analyses (multivariate HR for colon cancer-specific mortality, 1.34; 95% CI, 0.92-1.94).

Association between COX-2 expression and patient survival in various strata. We examined whether the effect of COX-2 expression on survival was modified by any of the clinical and molecular variables (Fig. 3). The effect of COX-2 overexpression on colon cancer-specific mortality was not significantly different across most strata of patient and disease characteristics. Notably, the effect of COX-2 overexpression was similar across the two independent cohort studies ($P_{interaction}$ = 0.87). We did observe an apparently significant modifying effect of p53 expression on the association between COX-2 and mortality ($P_{interaction}$ = 0.04). A significant adverse effect of COX-2 overexpression was present in p53-negative tumors but not among p53-positive tumors.

Table 3. Combined COX-2 and p53 status and patient survival in colon cancer

Combined COX-2/ p53 status	Total *n*	Colon cancer-specific mortality			Overall mortality		
		Deaths/ person-years	Univariate HR (95% CI)	Multivariate HR (95% CI)	Deaths/ person-years	Univariate HR (95% CI)	Multivariate HR (95% CI)
COX-2(-) p53(-)	91	16/844	1 (reference)	1 (reference)	37/844	1 (reference)	1 (reference)
COX-2(-) p53(+)	22	5/197	1.30 (0.48-3.55)	2.58 (0.89-7.48)	9/197	1.04 (0.50-2.16)	1.52 (0.71-3.24)
COX-2(+) p53(-)	313	85/2,689	1.61 (0.95-2.75)	2.09 (1.19-3.65)	134/2,689	1.13 (0.79-1.63)	1.35 (0.92-1.98)
COX-2(+) p53(+)	231	56/2,089	1.41 (0.81-2.46)	2.20 (1.20-4.03)	102/2,089	1.12 (0.77-1.63)	1.40 (0.93-2.11)
COX-2(+) total	544	141/4,778	1.53 (0.91-2.56)	2.12 (1.23-3.65)	236/4,778	1.12 (0.79-1.59)	1.37 (0.95-1.98)

NOTE: The multivariate analysis model includes age at diagnosis, year of diagnosis, sex, tumor location, stage, grade, and status of *KRAS, BRAF*, MSI, and CIMP. p53 status was determined by immunohistochemistry.

Combined COX-2 and p53 status and patient survival. Because there was evidence for effect modification by p53 status on the association between COX-2 and patient survival, we stratified tumors by combined status of COX-2 and p53 (Table 3). Compared with tumors that were negative for both COX-2 and p53, COX-2-positive tumors (regardless of p53 status) were associated with a significant increase in cancer-specific mortality (multivariate HR, 2.12; 95% CI, 1.23-3.65). At the same time, p53 status had little effect on mortality among COX-2-positive tumors.

Discussion

We conducted this study to examine the influence of COX-2 expression on outcome of colon cancer patients. We have found that COX-2 overexpression appears to predict an inferior cancer-specific survival independent of various clinical and molecular variables. The adverse effect of COX-2 overexpression was consistent across most strata of patient and tumoral characteristics, particularly across the two independent cohort studies. Our data support an adverse effect of COX-2 overexpression on survival of colon cancer patients.

Considerable experimental evidence supports a role of COX-2 in colorectal carcinogenesis (2). Randomized, placebo-controlled trials have uniformly shown that selective COX-2 inhibitors prevent adenoma recurrence among patients with a prior history of adenoma (5, 6). COX-2, possibly through production of inflammatory prostaglandins, may regulate angiogenesis, apoptosis, or tumor cell invasiveness (2, 40). We have shown previously that aspirin use decreases a risk for colon cancers that are positive for COX-2 but not a risk for COX-2-negative cancers, providing additional evidence for a role of COX-2 in colon carcinogenesis (25).

Studying molecular alterations and clinical outcome is important in cancer research (41–48). Our data support a role of COX-2 in determining biological behavior of colon cancer. COX-2 has been examined as a predictive biomarker in cancer (3, 8, 9). Previous studies are conflicting regarding prognostic significance of COX-2 in colorectal cancer with some (3, 8, 9) supporting and others (4, 10–16) refuting independent adverse effect of COX-2. These discrepant results are likely due to differences in patient cohorts, COX-2 detection methods, criteria for COX-2 overexpression, and multivariate survival analysis models. Our current study has comprehensively examined the effect of COX-2 on patient survival independent of clinical characteristics and other molecular events, including statuses of p53 alterations, mutations in *KRAS* and *BRAF*, MSI, and CIMP. All of these molecular events are potential confounders for the association between COX-2 and patient survival.

The relationship between COX-2 overexpression and p53 alteration has been examined previously. In one *in vivo* study, inhibition of COX-2 by celecoxib led to p53 activation in colon cancer cells (22). In other studies, COX-2 expression was inhibited by wild-type p53 in murine embryo cell lines (17), whereas COX-2 overexpression was induced by p53 and nuclear factor-κB in esophageal and colon cancer cells (23). It may be possible that COX-2 and p53 regulate each other to form a feedback loop. Thus, it may not be surprising to find a significant interactive effect of COX-2 and p53 alterations on patient survival. This possible interaction of COX-2 and p53 alterations needs to be further examined and confirmed by future studies.

Our study has several advantages including a large number of colon cancers in the two prospective cohort studies with adequate follow-up as well as extensive data on disease characteristics and other important tumoral molecular events. Thus, we have been able to show an effect of COX-2 on patient survival independent of clinical and other tumoral predictors of clinical outcome.

As a limitation of this study, data on cancer treatment are limited in our cohorts. Nonetheless, it is unlikely that chemotherapy use differed according to tumoral COX-2 status, especially because such data were not available to patients or treating physicians. In addition, beyond cause of mortality, data on cancer recurrences were not available in these cohorts. Nonetheless, given the median survival for metastatic colon cancer was ~10 to 12 months during much of the period of this study, colon cancer-specific survival should be a reasonable surrogate for cancer-specific outcomes. Despite the apparent effects of COX-2 expression on colon cancer-specific mortality, the influence of COX-2 on all-cause mortality was considerably attenuated. This is likely due to deaths unrelated to colon cancer in our cohort studies.

There is variability in grading COX-2 expression. Presently, there is no widely accepted standardized classification scheme. False-positive and false-negative results are well-known problems in immunohistochemistry. Nonetheless, previous studies have shown that Western and Northern blot analyses highly correlate with immunohistochemical expression of COX-2 (49), and our classification of COX-2 overexpression resulted in a similar proportion of COX-2-overexpressing tumors as other investigators (3, 4, 8–16). Moreover, we assessed COX-2 overexpression through central, blinded review of tumor specimens with rigorous comparison with internal controls with the substantial interobserver agreement (92%; $\kappa = 0.62$). Our COX-2 expression data in relation to MSI and CIMP are in agreement with studies by other investigators (18, 19, 50). Finally, any random misclassification of COX-2 status would have conservatively biased our results toward finding no significant difference in patient survival according to tumoral COX-2 expression.

In conclusion, this large prospective study of colon cancer patients suggests that COX-2 up-regulation is independently associated with a worse colon cancer-specific mortality. In addition, when compared with patients with tumors negative for both COX-2 and p53, patients with tumors positive for COX-2 exhibit longer survival regardless of p53 status. Our finding that COX-2 overexpression is associated with poor patient outcome may have significant clinical implications, considering an emerging role of COX-2 and its pathway as chemotherapeutic and chemopreventive targets.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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