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Expression of cyclooxygenase-2 (*COX-2*) mRNA in human colorectal adenomas

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Abstract

Cyclooxygenase-2 (COX-2) is an important target for the suppression of colorectal tumorigenesis by non-steroidal anti-inflammatory drugs (NSAIDs). To evaluate the role of *COX-2* in human sporadic colorectal adenomas, *COX-2* mRNA expression was examined by reverse transcription-polymerase chain reaction (RT-PCR) in 63 adenomas. *COX-2* mRNA was detected in all the adenomas at higher levels than in normal colorectal mucosa ($P < 0.001$). Levels of expression in the adenomas were correlated with their size ($P = 0.019$), but no relationships were demonstrable between *COX-2* expression and adenoma location, macroscopically observed configuration or microscopic degree of dysplasia. These findings suggest that *COX-2* plays an important role in the growth of sporadic colorectal adenomas.

Keywords: mRNA expression; Reverse transcription-polymerase chain reaction; Clinicopathological correlation

1. Introduction

Much interest has focused on the hypothesis that non-steroidal anti-inflammatory drugs (NSAIDs) suppress the development certain gastrointestinal tumours, particularly colorectal carcinomas [1]. Epidemiological evidence indicates an inverse relationship between the use of NSAIDs and colorectal cancer [2]. A decrease in the mean number of polyps and in their mean diameter was demonstrated in patients with familial adenomatous polyposis (FAP) treated with NSAIDs [3]; not only polyps in FAP and colorectal cancers, but also sporadic adenomas have been shown to be less likely among patients who consumed NSAIDs [4,5]. The precise mechanism underlying these effects remains to be clarified, but appears to depend on the blockade of COX-2 [1].

COX is a key regulatory enzyme in prostaglandin biosynthesis. Two isoenzymes have been identified [6]. While COX-1 is apparently expressed constitutively in virtually all mammalian tissues, COX-2 expression is almost undetectable under physiological conditions in most tissues, but can be induced by proinflammatory agents, growth factors and mitogens [6,7]. A high level of COX-2 expression is present in human colorectal cancers [8–13]. Studies using *in vitro* model systems showed cells overexpressing COX-2 to produce prostaglandins that stimulate angiogenesis, inhibit apoptosis, and increase the metastatic potential [6,14].

Colorectal adenomas are currently considered the precursor lesions for most colorectal cancers. The finding in the National Polyp Study that after removal of polyps there was a lower incidence of colorectal cancers than expected, supports this hypothesis [15]. In animal models, COX-2 expression is detected in 80–90% of colorectal adenocarcinomas and in 40–50% of adenomas [16]; induction of COX-2 is a very early event in the sequence of polyp formation and development [17]. Specific inhibition of COX-2 caused a greater reduction in polyp number than sulindac, which inhibits both COX-1 and -2 [17]. Since expression of COX-2 in sporadic human colorectal adenomas has been incompletely investigated, we analysed the relationship of COX-2 expression in these adenomas to various clinicopathological features.

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2. Patients and methods

We studied a total of 63 randomly chosen polyp specimens that had been removed from patients treated at our institution between January and October 1999. Fifty-eight polyps from 38 patients (mean polyp number 1.5 per patient; range 1–6) were excised completely by endoscopic polypectomy; five other polyps from 4 patients were obtained from surgically-resected specimens. No patient had a history of regular use of NSAID. The maximum diameter of a polyp was measured immediately after removal, prior to fixation. Macroscopically-evident configuration, maximum diameter and the location of the polyps were recorded. Each polyp was cut longitudinally through the stalk into halves. One-half of the polyp was frozen in liquid nitrogen and stored at −80°C until the extraction of mRNA, while the other was fixed in 10% buffered formalin and embedded in paraffin for routine histological examination. All 63 polyps examined for mRNA were confirmed histologically to be adenomas. All polyps represented sporadic occurrences; adenomas from FAP patients were excluded. For comparisons of *COX-2* mRNA levels, 47 samples of normal mucosa were collected randomly; 43 from the edge of resected specimens during surgery for colorectal cancer and four from endoscopically obtained normal-appearing mucosa.

Total RNA was extracted according to the method of Chomczynski [18] using the RNeasy kit (Qiagen, Chatsworth, CA, USA) as previously described in Ref. [9]. Total RNA was quantitated based on absorbance readings at 260 nm. Reverse transcription (RT) was carried out using total RNA and the SuperScript reverse transcriptase (GIBCO BRL, Gaithersburg, MD, USA) to obtain cDNA.

We designed primers for polymerase chain reaction (PCR) to coamplify both *COX-1* and *COX-2* mRNA in the same tube for each sample; the antisense primer was shared for the amplification of *COX-1* and *COX-2* mRNA. The primers used were 5′-TGC CCA GCT CCT GGC CCG CCG CTT-3′ (*COX-1* sense; bases 522 to 545), 5′-AGT CAA AGA TAC TCA GGC AGA-3′ (*COX-2* sense; bases 851–872), and 5′-GYA GYT CTG GGT CAA ATT TCA G-3′ (common antisense; bases 1080–1101 for *COX-1* and 1133–1154 for *COX-2* with Y indicating an equimolar mixture of T and C), as previously described in Ref. [9]. Three amounts of cDNA ranging from 20 to 100 ng were amplified for each sample. PCR products were electrophoresed in 1.5% agarose gels and visualised by ethidium bromide straining. Densities of the bands were quantified with the Multi-Analyst 1.0.2 software package (Bio-Rad Laboratories, Hercules, CA, USA). Optimal cDNA amounts for PCR amplification conditions were chosen from the three amounts in a linear range for each sample. Since *COX-1* is expressed constitutively in all tissues as a housekeeping gene, a *COX-2* index was calculated as the band density ratio of *COX-2* to that of *COX-1* to estimate the *COX-2* mRNA expression [9].

Macroscopic configurations of adenomas were classified into three types: pedunculated, semipedunculated and sessile. By the maximum diameter of the unfixed specimens, adenoma size was classified as small ($\varnothing \leqslant 6$ mm), medium (7 mm $< \varnothing \leqslant 12$ mm) or large (13 mm $< \varnothing$). Adenomas were classified by location as from the right colon, left colon or rectum. Degree of dysplasia of each adenoma was graded microscopically as mild, moderate or severe according to the World Health Organization (WHO) classification. Intramucosal cancers were included among adenomas with severe atypia. *COX-2* index was analysed in terms of the relationships to clinicopathological findings in the adenomas.

Statistical significance was determined by the Chi-square analysis, the Mann–Whitney test, the Kruskal–Wallis test, the one-factor ANOVA, the Fisher's PLSD (*post hoc*) test, or simple regression and Spearman's coefficient by rank using the Stat View 4.5 software package (Abacus Concepts, Berkeley, CA, USA). *P* values less than 0.05 were interpreted as indicating statistical significance. All results are shown as mean ± standard error (S.E.).

3. Results

Clinicopathological findings for the 63 adenomas are listed in Table 1. Sizes of adenomas varied from 3 to 25 mm in maximum diameter (mean 10 mm). Adenoma size was significantly correlated with the degree of dysplasia ($P = 0.025$, Table 2). *COX-1* mRNA was detected in all adenomas and normal mucosal samples. *COX-2* mRNA expression was expressed relative to that of

Table 1
Clinicopathological findings for 63 adenomas

Clinicopathological factors	Number (%)
Location	
Right colon	35 (56)
Left colon	16 (25)
Rectum	12 (19)
Size	
Small	20 (32)
Medium	29 (46)
Large	14 (22)
Configuration	
Pedunculated	22 (35)
Semipedunculated	28 (44)
Sessile	13 (21)
Grade of dysplasia	
Mild	29 (46)
Moderate	20 (32)
Severe	14 (22)

COX-1 (*COX-2* index, representing the *COX-2*/*COX-1* band density ratio). *COX-2* mRNA was detected in only 35% of normal mucosal samples, and only at a very low level. In contrast, all adenomas showed *COX-2* mRNA expression and *COX-2* indices in the adenomas were significantly higher than that in normal mucosa (1.67 ± 0.17 versus 0.28 ± 0.07; $P < 0.001$ by the Mann–Whitney test, Fig. 1). Interestingly, positive correlation was found between the *COX-2* indices and the size of adenomas by simple regression and Spearman's coefficient by rank ($R = 0.34$, $P = 0.007$; Fig. 2a). The *COX-2* indices in small, medium and large adenomas were 1.08 ± 0.11, 1.66 ± 0.29 and 2.54 ± 0.45, respectively. Although significant elevation even in the smallest adenomas beyond normal mucosal expression was observed ($P < 0.001$ by the Mann–Whitney test), the *COX-2* index was significantly higher in adenomas with the larger diameters ($P < 0.001$, by the one factor ANOVA; Fig. 2b). By the Fisher's PLSD test, the P values between normal mucosa and small adenomas, small and medium adenomas, medium and large adenomas were 0.006, 0.067 and 0.015, respectively.

In contrast, there were no correlations between *COX-2* mRNA expression levels in adenomas and location, configuration, or degree of dysplasia ($P = 0.38$, 0.38 and 0.36, respectively; Figs. 3a–c).

Table 2
Relationship between size and dysplasia in 63 adenomas

Dysplasia	Size			Overall
	Small ($n = 20$) (%)	Medium ($n = 29$) (%)	Large ($n = 14$) (%)	
Mild	14 (70)	13 (45)	2 (14)	29 (46)
Moderate	4 (20)	10 (34)	6 (43)	20 (32)
Severe	2 (10)	6 (21)	6 (43)	14 (22)

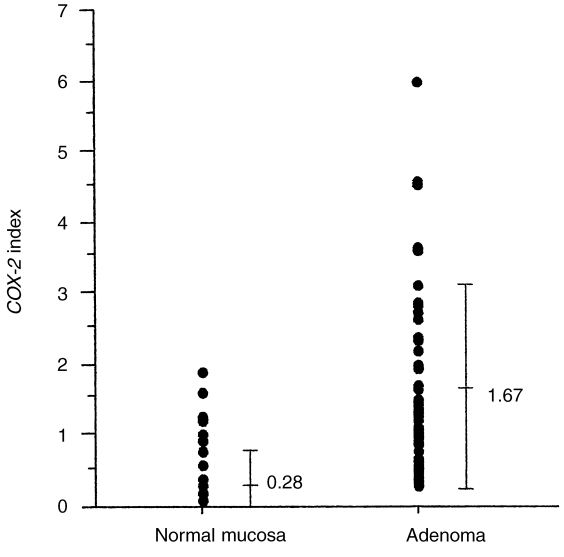

Fig. 1. Comparison of *COX-2* mRNA expression between 63 adenomas and 47 normal mucosal samples. *COX-2* mRNA expression levels were expressed as *COX-2* indices (representing the *COX-2*/*COX-1* band density ratio). The number and vertical bar to the right of each size group indicate the mean and standard deviation (S.D.) of the *COX-2* index. Adenomas showed significantly higher *COX-2* indices than normal colorectal mucosal samples.

4. Discussion

In the present study, we demonstrated that *COX-2* mRNA expression was markedly increased in sporadic colorectal adenomas compared with normal colorectal mucosa. Moreover, *COX-2* mRNA expression was significantly greater in adenomas with larger diameters.

Other recent studies have also examined *COX-2* mRNA expression in human sporadic colorectal adenomas; Eberhart and colleagues [10] found a marked excess in *COX-2* mRNA expression in six of 14 adenomas compared with paired normal mucosal samples. Maekawa and colleagues [13] reported an enhanced *COX-2* mRNA expression in nine of 10 adenomas compared with the accompanying normal mucosa. However, these studies did not examine relationships between *COX-2* mRNA expression in sporadic colorectal adenomas and specific clinicopathological findings.

The present study demonstrated a correlation between *COX-2* mRNA expression and the size of human sporadic colorectal adenomas. In contrast, location, configuration and degree of dysplasia had no relationship to *COX-2* mRNA expression in the adenomas. Interestingly, Hao and colleagues [19] semiquantitatively estimated by immunohistochemistry the extent and intensity of COX-2 protein staining in 85 adenomas. Although these authors concluded that COX-2 expression was unrelated to the size of sporadic colorectal adenomas, five of 16 adenomas (31%) 0.5 cm or less in diameter and 47 of 69 adenomas (68%) larger than 0.5 cm showed strong COX-2 protein staining in their study.

Synthesis of COX-2 protein parallels an increase after stimulation with mitogens or a tumour promoter [6]. Yang and colleagues [20] have reported that the levels of prostanoids in adenomas of FAP patients were elevated in a size-dependent manner; they did not observe an elevation of prostanoids until an adenoma reached a diameter of approximately 6–7 mm. However, in the present study, we found a significant increase of *COX-2* mRNA expression even in small adenomas measuring 6 mm or less in diameter compared with normal mucosa. The reasons for this discrepancy are not clear, but differences in design including the tissues used (FAP-related adenomas versus sporadic adenomas) and the variables examined (prostaglandin levels versus *COX-2* mRNA expression) may have led to the differing findings in these studies.

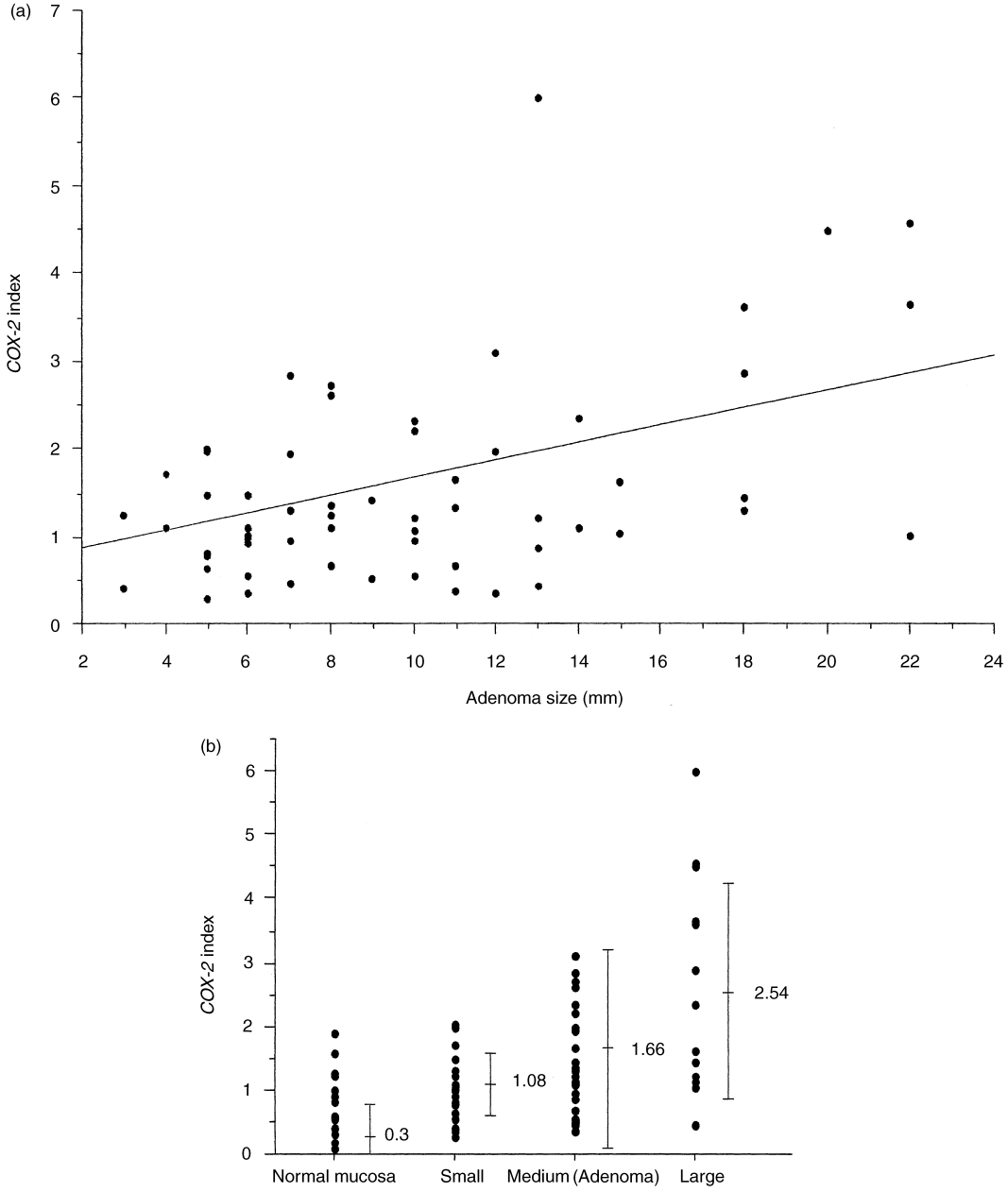

Fig. 2. (a) Positive correlation is found between the *COX-2* index and size of adenomas by the simple regression and Spearman's coefficient by rank ($R = 0.34$, $P = 0.007$). (b) Size-dependent increase in *COX-2* index levels in colorectal adenomas. Sizes of adenomas are shown in three classes: small ($\varnothing \leqslant 6$ mm), medium (7 mm $\leqslant \varnothing \leqslant$ 12 mm) and large (13 mm $\leqslant \varnothing$).

The relationship between *COX-2* expression and location or macroscopic configuration of sporadic colorectal adenomas still needs to be clarified. Recently, Chapples and colleagues [21] showed that superficial interstitial cell *COX-2* expression in adenomas distal to the splenic flexure were increased significantly compared with adenomas proximal to the splenic flexure. However, these investigators found no relationship between the adenoma site and *COX-2* expression in the deep interstitial cells of adenomas. Interstitial cells were not separately examined in our study because of mRNA extraction. Dimberg and colleagues [12] noted a greater overexpression of the COX-2 protein in cancers located in the rectum than elsewhere in the colon. They postulated that this difference reflected local variations in gene regulatory factors affecting COX-2 expression or could have resulted from regional differences in tumour progression. In contrast, other investigators [11,13], including ourselves [9], found no correlation between *COX-2* expression and the location of colorectal carcinomas. Similarly our conclusion regarding location of colorectal carcinoma, we found COX-2 mRNA

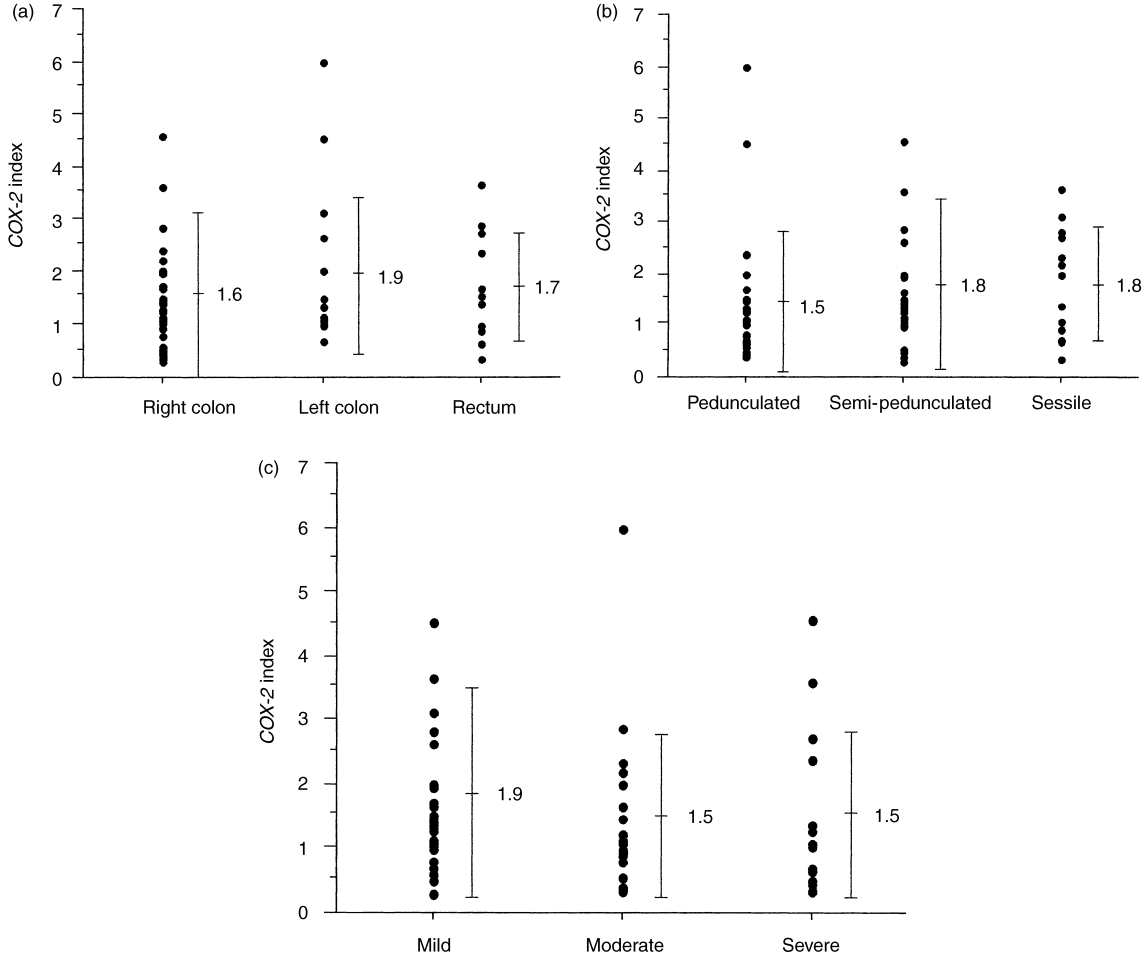

Fig. 3. Relationships between the *COX-2* index levels and clinicopathological findings of adenomas. No correlation with a; location, b; configuration, or c; grade of dysplasia was observed ($P = 0.38$, 0.38 and 0.36, respectively, by the Kruskal–Wallis test).

expression to show no correlation with location in colorectal adenomas.

Adenoma size is reported to be a major risk factor associated with high-grade dysplasia [22]. We have confirmed that the size of the adenoma was significantly correlated with the degree of dysplasia. *COX-2* expression correlated well with adenoma size, but not with the degree of dysplasia in our study. Chapples and colleagues [21] also showed by immunohistochemistry that the degree of epithelial cell dysplasia did not predict COX-2 protein expression in human colorectal adenomas. Matsuhashi and colleagues [23] reported that 13 of the 20 sporadic colorectal adenomas shrank or disappeared, but the degree of atypia was not affected after treatment with sulindac. These findings suggest that COX-2 is involved in important events in colorectal adenoma growth, but may not influence the degree of dysplasia in the adenomas. COX-2 seems to play an important role in colorectal tumorigenesis especially in the growth of adenoma. Increased metabolic activity of COX-2 and elevation of prostanoids resulting from an increase in the *COX-2* mRNA level may contribute to the growth of adenomas [20].

In conclusion, our observations support the hypothesis that increased *COX-2* expression is involved in tumorigenesis, not only in carcinomas, but also in sporadic polypoid colorectal adenomas. Moreover, a significant correlation between *COX-2* expression and the size of the adenomas strongly suggests that *COX-2* may play an important role in the growth of these adenomas.

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