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Cyclo-oxygenase and Colon Cancer: Clues to the Aspirin Effect?

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Epidemiological and experimental studies indicate an inverse relationship between the risk of colon cancer development and intake of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). All NSAIDs are known inhibitors of cyclo-oxygenase, the enzyme responsible for converting arachidonic acid to prostaglandins. Prostaglandins have been implicated in the pathogenesis of colon cancer and it has been suggested that the preventive effect of NSAIDs is due to inhibition of cyclo-oxygenase activity. Cyclo-oxygenase exists in two different isoforms, cyclo-oxygenase-1 and cyclo-oxygenase-2, and data obtained during the last few years have suggested that cyclo-oxygenase-2 might be involved in both human and experimental colon carcinogenesis. The purpose of this review is to provide an update on recent studies regarding cyclo-oxygenase, in particular cyclo-oxygenase-2, in relation to colon cancer in humans and in experimental models.

Key words: colon cancer; cyclo-oxygenase; nonsteroidal anti-inflammatory drugs.

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Aspirin and Prevention of Colon Cancer

Aspirin, and other nonsteroidal anti-inflammatory drugs (NSAIDs) (e.g. sulindac, indomethacin and piroxicam) are among the most commonly used medications worldwide due to their anti-inflammatory and analgesic effects.

Epidemiological studies have demonstrated an association between regular long-term consumption of NSAIDs, in particular aspirin, and a reduced incidence and mortality in colon cancer (1–8). Moreover, sulindac (9–11), and recently indomethacin (12), have been shown to reduce the number and size of adenomatous colonic polyps in patients with familial adenomatous polyposis (FAP). In line with the findings in humans, animal studies have shown that NSAIDs may prevent tumour development in the colon of rodents exposed to various carcinogens (13–19). In a recent study it was found that piroxicam prevented development of tumours in *Min* mice, used as a model for studies on FAP because of their particular ability to spontaneously develop intestinal adenomas (20).

Taken together, results obtained from epidemiological and experimental studies clearly indicate an inverse relation between colon cancer and treatment with NSAIDs. However, the precise mechanisms by which NSAIDs affect colon carcinogenesis have not yet been clarified.

Cyclo-oxygenase

The NSAIDs are known to bring about most of their anti-inflammatory activities by interfering with the enzymatic activity of cyclo-oxygenase, thereby reducing the formation of arachidonic acid metabolites, in particular prostaglandins. Arachidonic acid is released from cellular phospholipids by the enzyme phospholipase A_2 (21), and cyclo-oxygenase then catalyses the formation of an endoperoxide intermediate, prostaglandin H_2 (PGH_2), from arachidonic acid (22). PGH_2 is further metabolized by various prostaglandin synthethases to form specific prostaglandins (22).

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Cyclo-oxygenase exists in two genetically different isoforms, cyclo-oxygenase-1 (COX-1) (23) and cyclo-oxygenase-2 (COX-2) (24, 25), which are likely to represent two, at least partly independent prostaglandin biosynthetic systems. COX-1 and COX-2 seem to be coexpressed at low but detectable levels in most cell-types and tissues (24, 26–29), including the human colon (30). In general, however, COX-1 seems to be the isoenzyme that is constitutively expressed and therefore most important for the production of prostaglandins during basal conditions, whereas COX-2 seems to be responsible for the increased production in response to various cytokines, mitogens and growth factors (24, 25, 27–29, 31, 32) in pathological conditions, such as inflammation. Accordingly, the expression of COX-2 is inhibited by anti-inflammatory glucocorticoids (33, 34). In line with these assumptions, a recent study shows that prostaglandins produced and required for normal physiological functioning in the gastrointestinal tract are derived from the COX-1 isoform (35). A schematic presentation of the regulation and role of cyclo-oxygenase in prostaglandin formation is outlined in Figure 1.

Inhibition of Cyclo-oxygenase by NSAIDs

All NSAIDs are well-known inhibitors of cyclo-oxygenase activity (36, 37), although there are differences between them regarding their relative inhibition of COX-1 and COX-2 (34, 38). Aspirin, however, is unique in that it irreversibly modifies the active site of cyclo-oxygenase whereas the other NSAIDs compete with arachidonic acid for the active site of the isoenzymes (34). Moreover, whereas treatment with aspirin completely inhibits the activity of COX-1, treatment of COX-2 changes the catalytic site of the enzyme and 15R-hydroxy-5,8,11,13-eicosatetraenoic acid (15R-HETE) is formed instead of PGH_2 (38) (Fig. 1).

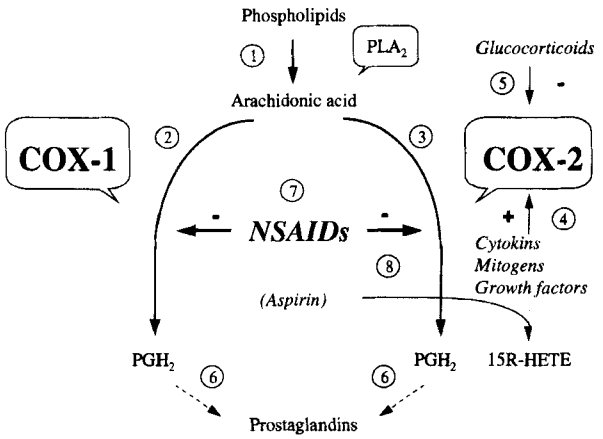

Figure 1. Regulation and role of cyclo-oxygenase in prostaglandin formation. 1: Arachidonic acid is released from membrane phospholipids by phospholipase A_2 (PLA_2), and 2: then converted to endoperoxide intermediate prostaglandin H_2 (PGH_2) by either the constitutive cyclo-oxygenase, cyclo-oxygenase-1 (COX-1), or 3: by cyclo-oxygenase-2 (COX-2). 4: COX-2 is induced and activated by inflammatory and mitogenic agents and 5: inhibited by glucocorticoids. 6: Specific prostaglandin synthethases convert PGH_2 into different prostaglandins. 7: nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the conversion of arachidonic acid to PGH_2 by COX-1 and/or COX-2. 8: Aspirin, in addition, changes the catalytic activity of COX-2; arachidonic acid is metabolized to 15R-HETE.

The anti-inflammatory activity of NSAIDs is thought to arise from inhibition of COX-2, whereas the well-known serious gastrointestinal side-effects, such as gastric ulceration and bleeding, may be associated with the inhibition of COX-1 (33, 39). Specific COX-2 inhibitors have recently been developed and have potent anti-inflammatory properties with reduced risk of gastrointestinal side-effects (33, 39). Specific inhibitors of COX-2 might thus become useful alternatives to the commonly used unselective NSAIDs.

Prostaglandins and Colon Cancer

It is generally thought that prostaglandin E_2 (PGE_2) participates in colon carcinogenesis (16, 40–43) because both human (40–42) and experimental colonic tumours (16, 43) produce increased amounts of this particular prostaglandin. Although the role of PGE_2 in colon carcinogenesis remains unclear, it has been suggested to involve modulation of cellular and humoral immune responses (44–46) and effects on intestinal epithelial proliferation (47, 48).

Whatever the precise role of PGE_2, results from different studies have suggested that PGE_2 might be involved early in the progress of carcinogenesis. The concentration of PGE_2 is increased in noncancerous colonic adenomas in humans (30, 49), in macroscopically normal colon of rodents exposed to colonic carcinogens (16, 43, 50, 51) and in macroscopically normal bowel in *Min* mice (52). Also *in vitro* production of prostaglandins from arachidonic acid was found to be increased in the normal mucosa of rats exposed to carcinogen (53). Moreover, the apparent lack of association between the PGE_2 level and Dukes' stage of colon cancer further supports the hypothesis that PGE_2 is involved during the early steps of colon cancer development (41).

Cyclo-oxygenase Expression and Colon Cancer

The increased concentration of PGE_2 in human and rodent colonic cancers indicates that colon cancer development is associated with an increased cyclo-oxygenase activity. Of special interest is, therefore, to investigate which particular isoform of cyclo-oxygenase is responsible for the increased PGE_2 production during colon carcinogenesis.

Recent studies on human colorectal cancers indicate that the expression of COX-2 messenger ribonucleic acid (mRNA) and protein is increased in the tumours compared with matched macroscopically normal colonic mucosa in the same patients. In one study, the expression of COX-2 protein was elevated in all colon cancer specimens from 15 patients (54). In another study, COX-2 protein was detected in 19 of 25 colon cancer tumours but only in two of 25 matched non-tumour colon specimens (55). A marked increase of COX-2 mRNA was found in 12 of 14 human colonic carcinomas (30). This finding was confirmed by two other studies in which the expression of COX-2 mRNA was increased in five of five (56) and in 14 of 15 (57) human colon cancers from different patients (Fig. 2, left-hand panel). Furthermore, COX-2 mRNA was increased in 30 of 35 experimentally induced colonic tumours in rats compared with macroscopically normal colon distant from the tumours (57) (Fig. 2, right-hand panel). Another study showed an increased level of COX-2 mRNA in five of six chemically induced tumours and the level of COX-2 protein was increased in four of the tumours (58). Moreover, it has been shown that intestinal tumours in *Min* mice have increased levels of both COX-2 mRNA and COX-2 protein compared with adjacent normal intestinal mucosa (59).

The upregulation of COX-2 mRNA in human colon carcinomas does not seem to be associated with Dukes' stage (30, 55, 57), suggesting an early role of COX-2 during colon carcinogenesis. This is supported by the finding that COX-2 mRNA was increased in specimens from the macroscopically normal colon of rats receiving carcinogen, compared with colonic specimens from saline-treated control rats (57) (Fig. 2, right-hand panel). Furthermore, the content of COX-2 mRNA is increased already in the macroscopically normal intestine of *Min* mice compared with littermates not bearing the mutation responsible for the spontaneous development of adenomas (52).

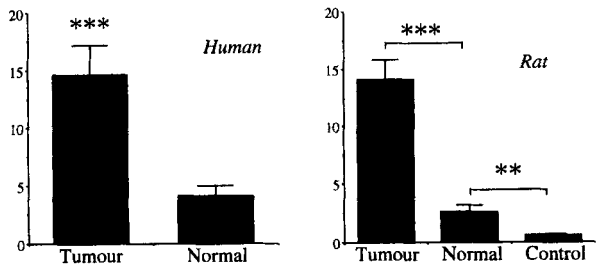

Figure 2. Cyclo-oxygense-2 mRNA content in human colon tumours and in chemically induced colon tumours from rats (Tumour), in comparison with matched normal colonic specimens (Normal) or colonic specimens from untreated control rats (Control). The human tissues included 15 tumours and paired normal mucosa from 15 people, and the rat tissues included 35 tumours and 19 matched normal samples from 19 rats, and 17 control specimens from 17 different rats. Comparative statistical analysis was made with Wilcoxon's rank sum test for unpaired observations (rat) or with Wilcoxon's signed rank test for paired observations (human). $^{**}P<0.01$; $^{***}P<0.001$. Adapted from (57).

Two different studies indicate that the increased expression of COX-2 in human colon cancer originates mainly from the neoplastic epithelial cells, whereas little or no COX-2 is expressed by the normal colonic epithelium (54, 56). Also in *Min* mice, the increased COX-2 expression was localized to the dysplastic or neoplastic epithelial cells, whereas no expression was detected in normal mucosal cells (59). Studies in experimental cell systems indicate that COX-2 is transcribed abnormally in human colon cancer cells leading to an increased COX-2 expression in these cells (56). It is suggested that this is caused by a substance yet unidentified over-expressed by the cancer cells. It is thus possible that the increased expression of COX-2 in human and experimental colon carcinogenesis is due to an abnormal transcription of the COX-2 gene of the colonic epithelium.

In contrast to COX-2, the concentrations of COX-1 protein and mRNA seem unchanged in colonic tumours (30, 54, 56–58) or decreased as demonstrated in one study (55). Although a small but significant increase was found also in the COX-1 mRNA content of experimentally induced tumours, this increase was much less pronounced than the corresponding increase in COX-2 mRNA (57).

Altogether, available data from studies on human and experimental colon cancer indicate that COX-2 is the isoform responsible for the increased PGE_2 production during colon carcinogenesis.

Cyclo-oxygenase Inhibition by NSAIDs in Relation to Colon Carcinogenesis

The role of COX-2 leading to increased PGE_2 production in colon cancers implies that the effect of NSAIDs on colon carcinogenesis is mediated by inhibition of COX-2. However, only a few studies have been published so far in which the antineoplastic effect of NSAIDs in relation to COX-2 has been investigated.

Apoptosis is important for the maintenance of rapid turnover of the intestinal epithelium and a decreased apoptotic activity of the colonic epithelium may result in prolonged survival of abnormal cells which in turn may lead to colon carcinogenesis. *Min* mice exhibit a decreased fraction of intestinal epithelial cells undergoing apoptosis but treatment with sulindac restored it to normal levels (52). Moreover, treatment with sulindac also decreased the bowel content of COX-2 and PGE_2 to normal (52), suggesting that the effect of sulindac on apoptosis was due to COX-2 inhibition and decreased PGE_2 formation.

Aberrant crypt foci are recognized as early pre-neoplastic lesions in experimentally induced colon carcinogenesis and have been suggested as putative precursor lesions from which adenomas and carcinomas may develop in the colon (60). Several NSAIDs, including aspirin, have been shown to inhibit the formation of such lesions (13, 60, 61). Most interesting is that the COX-2-specific NSAIDs seem to be as potent inhibi-

tors as the nonselective ones suggesting that COX-2, rather than COX-1, may be involved in the formation of aberrant crypt foci (60, 61). The finding that sulindac inhibits tumour formation in *Min* mice almost completely and at the same time decreases the bowel content of COX-2 and PGE_2 to normal, further supports that inhibition of COX-2 is important in colon carcinogenesis and in the action of NSAIDs during colon carcinogenesis (52). Moreover, it was recently shown that treatment with a COX-2-specific inhibitor or disruption of the COX-2 gene suppresses the tumour formation in another mouse model of FAP (62). Indeed, this study provides the first direct piece of evidence of a relationship between COX-2 expression and polyp formation (62).

Intestinal epithelial cells constructed to permanently overexpress COX-2 protein may have features which enhance the tumourigenic potential (63, 64), including decreased apoptosis and decreased expression of certain growth factor receptors and adhesion molecules. Most interestingly, all these effects were reversed by sulindac (63), strongly supporting a role for COX-2 in colon carcinogenesis and for NSAID-mediated COX-2 inhibition in its prevention.

Taken together, these results indicate that colon carcinogenesis is associated with changes in COX-2 activity and inhibition of COX-2 activity may be responsible for the preventive effect of NSAIDs on colon cancer development. It is known, however, that NSAIDs may have other effects in addition to inhibition of cyclo-oxygenase and, therefore, may have effects on cancer development unrelated to cyclo-oxygenase activity. Moreover, it is possible that cyclo-oxygenase has effects on colon carcinogenesis which are unrelated to prostaglandin synthesis. One possibility is that cyclo-oxygenase is involved in the metabolic activation of potential carcinogens in the colon (65) and that the NSAIDs inhibit this activation. It has also been suggested that the antineoplastic effect of aspirin involves induced formation of 15R-HETE from the acethylated COX-2 (66) as 15R-HETE may be further metabolized (67) to compounds which have antiproliferative effects (66). Another possibility is that NSAIDs decrease the level of prostaglandins in the colon without affecting the cyclo-oxygenase activity by inhibiting the formation of lipid bodies, inducible cytoplasmic cellular inclusions which may participate in generating prostaglandins (68). Aspirin and other NSAIDs may inhibit lipid body formation independent of cyclo-oxygenase inhibition (68).

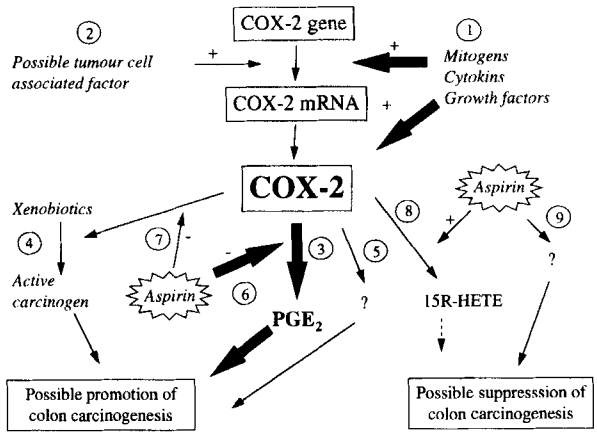

Figure 3. Schematic presentation of a hypothesized role of cyclo-oxygenase-2 (COX-2) and NSAIDs (exemplified by aspirin) in colon cancer. 1: Colon carcinogenesis is associated with an increased expression of COX-2 messenger ribonucleic acid (mRNA) and protein in the colonic epithelium, probably due to the action of various mitogens, cytokines and growth factors, or 2: possibly by the action of a cellular factor expressed by the transformed colonocyte. 3: The increased COX-2 activity causes an increased synthesis of prostaglandin E_2 which is likely to promote events involved in colon carcinogenesis. 4: The increased COX-2 activity may also result in activation of potentially xenobiotic carcinogens which may promote colon carcinogenesis. 5: The increased COX-2 activity may also promote colon carcinogenesis by some mechanisms yet unidentified. 6: Aspirin and other NSAIDs are likely to prevent colon carcinogenesis by inhibition of COX-2-mediated PGE_2 synthesis. 7: Aspirin may also inhibit colon carcinogenesis by inhibition of COX-2-mediated activation of carcinogens, 8: by inducing COX-2-mediated synthesis (note: only aspirin) of 15-HETE, or 9: perhaps by mechanisms independent of COX-2 activity.

COX-2 activation and formation of PGE_2 may be important events in the early steps of colon carcinogenesis. NSAIDs are inhibitors of cyclo-oxygenase activity and also well-documented inhibitors of human and experimental colon cancer. Thus, it seems likely that the inhibitory action of NSAIDs on colon carcinogenesis involves inhibition of COX-2, thereby preventing the formation of putative tumour-promoting prostaglandins in the colon. Although the few studies published so far support this hypothesis, more investigations are needed to clarify the role of COX-2 inhibition by NSAIDs in colon cancer. A schematic presentation of a hypothesized role of COX-2 and NSAIDs in colon cancer is outlined in Figure 3.

Summary and Conclusions

Evidence from studies on human colon cancer and on experimental colon cancer in rodents suggest that an increased COX-2 expression and PGE_2 formation is associated with cancer development. Moreover, these studies suggest that this increase precedes the development of malignant tumours, suggesting that

References

1. **Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC.** Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. *Ann Intern Med* 1994; 121: 241–6.
2. **Schreinemachers DM, Everson RB.** Aspirin use and lung, colon, and breast cancer incidence in a prospective study. *Epidemiology* 1994; 5: 138–46.

3. **Muscat JE, Stellman SD, Wynder EL.** Nonsteroidal anti-inflammatory drugs and colorectal cancer. *Cancer* 1994; 74: 1847–54.
4. **Kauppi M, Pukkala E, Isomäki H.** Low incidence of colorectal cancer in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 1996; 14: 551–3.
5. **Giovannucci E, Egan K, Hunter D, et al.** Aspirin and the risk of colorectal cancer in women. *N Engl J Med* 1995; 333: 609–14.
6. **Kune G, Kune S, Watson L.** Colorectal cancer risk, chronic illness, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. *Cancer Res* 1988; 48: 4399–404.
7. **Suh O, Mettlin C, Petrelli N.** Aspirin use, cancer, and polyps of the large bowel. *Cancer* 1993; 72: 1171–7.
8. **Thun M, Namboodiri M, Calle E, Flanders W, Heath C.** Aspirin use and risk of fatal cancer. *Cancer Res* 1993; 53: 1322–7.
9. **Rigau J, Pique J, Rubio E, Planas R, Tarrech J, Bordas J.** Effects of long-term sulindac therapy on colonic polyposis. *Ann Intern Med* 1991; 115: 952–4.
10. **Spagnesi M, Tonelli F, Dolara P, et al.** Rectal proliferation and polyp occurrence in patients with familial adenomatous polyposis after sulindac treatment. *Gastroenterology* 1994; 106: 362–6.
11. **Waddell W, Gasner G, Cerise E, Loughry R.** Sulindac for polyposis of the colon. *Am J Surg* 1989; 157: 175–9.
12. **Hirota C, Iida M, Aoyagi K, et al.** Effect of indomethacin suppositories on rectal polyposis in patients with familial adenomatous polyposis. *Cancer* 1996; 78: 1660–5.
13. **Mereto E, Frencia L, Ghia M.** Effect of aspirin on incidence and growth of aberrant crypt foci induced in the rat colon by 1,2-dimethylhydrazine. *Cancer Lett* 1994; 76: 5–9.
14. **Metzger U, Meier J, Uhlschmid G, Weihe H.** Influence of various prostaglandin synthesis inhibitors on DMH-induced rat colon cancer. *Dis Colon Rectum* 1984; 27: 366–9.
15. **Reddy BS, Tokumo K, Kulkarni N, Aligia C, Kelloff G.** Inhibition of colon carcinogenesis by prostaglandin synthesis inhibitors and related compounds. *Carcinogenesis* 1992; 13: 1019–23.
16. **Reddy BS, Rao CV, Rivenson A, Kelloff G.** Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. *Carcinogenesis* 1993; 14: 1493–7.
17. **Pollard M, Luckert P.** Effect of indomethacin on intestinal tumors induced in rats by the acetate derivative of dimethylnitrosamine. *Science* 1981; 214: 558–9.
18. **Reddy B, Maruyama H, Kelloff G.** Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal anti-inflammatory drug, during different stages of rat colon tumor development. *Cancer Res* 1987; 47: 5340–6.
19. **Skinner S, Penney A, Penney G, O'Brian P.** Sulindac inhibits the rate of growth and appearance of colon tumors in the rat. *Arch Surg* 1991; 26: 1094–6.
20. **Jacoby R, Marshall D, Newton M, et al.** Chemoprevention of spontaneous intestinal adenomas in the ApcMin mouse model by the nonsteroidal anti-inflammatory drug piroxicam. *Cancer Res* 1996; 56: 710–4.
21. **Kudo I, Murakami M, Hara S, Inoue K.** Mammalian non-pancreatic phospholipases A2. *Biochim Biophys Acta* 1993; 117: 217–31.
22. **Smith WL.** Prostanoid biosynthesis and mechanisms of action. *Am J Physiol* 1992; 263: F181–91.
23. **Yokoyama C, Tanabe T.** Cloning of human gene encoding prostaglandin endoperoxide synthase and primary structure of the enzyme. *Biochem Biophys Res Commun* 1989; 165: 888–94.
24. **Hla T, Neilson K.** Human cyclooxygenase-2 cDNA. *Proc Natl Acad Sci USA* 1992; 89: 7384–8.
25. **Jones DA, Carlton DP, McIntyre TM, Zimmerman GA, Prescott SM.** Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. *J Biol Chem* 1993; 268: 9049–54.
26. **O'Neill GP, Ford-Hutchinson AW.** Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. *FEBS Lett* 1993; 330: 156–60.
27. **Crofford LJ, Wilder RL, Ristimäki AP, et al.** Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. Effects of interleukin-1β, phorbol ester, and corticosteroids. *J Clin Invest* 1994; 93: 1095–101.
28. **Habib A, Créminon C, Frobert Y, Grassi J, Pradelles P, Maclouf J.** Demonstration of an inducible cyclooxygenase in human endothelial cells using antibodies raised against the carboxyl-terminal region of the cyclooxygenase-2. *J Biol Chem* 1993; 31: 23448–54.
29. **Rzymkiewicz D, Leingang K, Baird N, Morrison AR.** Regulation of prostaglandin endoperoxide synthase gene expression in rat mesangial cells by interleukin-1β. *Am J Physiol* 1994; 266: F39–45.
30. **Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, Dubois RN.** Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994; 107: 1183–8.
31. **Gustafson-Svärd C, Lilja I, Sjödahl R, Tagesson C.** Cytosolic phospholipase A2 and cyclooxygenase-2 mediate release and metabolism of arachidonic acid in tumor necrosis factor-α-primed cultured intestinal epithelial cells (INT 407). *Scand J Gastroenterol* 1995; 30: 1000–7.
32. **DuBois RN, Tsujii M, Bishop P, Awad JA, Makita K, Lanahan A.** Cloning and characterization of a growth factor-inducible cyclooxygenase gene from rat intestinal epithelial cells. *Am J Physiol* 1994; 266: G822–7.
33. **Masferrer JL, Zweifel BS, Manning PT, et al.** Selective inhibition of inducible cyclooxygenase 2 *in vivo* is anti-inflammatory and nonulcerogenic. *Proc Natl Acad Sci USA* 1994; 91: 3228–32.
34. **Vane J, Botting R.** Mechanism of action of anti-inflammatory drugs. *Scand J Rheumatol* 1996; 25: 9–21.
35. **Kargman S, Charleson S, Cartwright M, et al.** Characterization of prostaglandin G/H synthase 1 and 2 in rat, dog, monkey, and human gastrointestinal tracts. *Gastroenterology* 1996; 111: 445–54.
36. **Earnest DL, Hixson LJ, Alberts DS.** Piroxicam and other cyclooxygenase inhibitors. Potential for cancer chemoprevention. *J Cell Biochem* 1992; Supplement 161: 156–66.
37. **Marnett LJ.** Aspirin and the potential role of prostaglandins in colon cancer. *Cancer Res* 1992; 52: 5575–89.
38. **Meade EA, Smith WL, DeWitt DL.** Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *J Cell Biol* 1993; 268: 6610–4.
39. **Klein T, Nüsing R, Pfeilschifter J, Ullrich V.** Selective inhibition of cyclooxygenase 2. *Biochem J* 1994; 48: 1605–10.
40. **Bennett A, Civier A, Hensby CN, Melhuish PB, Stamford IF.** Measurement of arachidonate and its metabolites extracted from human normal and malignant gastrointestinal tissues. *Gut* 1987; 28: 315–8.
41. **Rigas B, Goldman IS, Levine L.** Altered eicosanoid levels in human colon cancer. *J Lab Clin Med* 1993; 122: 518–23.
42. **Hendrickse CW, Kelly RW, Radley S, Donovan IA, Keighley MRB, Neoptolemos JP.** Lipid peroxidation and prostaglandins in colorectal cancer. *Br J Surg* 1994; 81: 1219–23.
43. **Yamaguchi A, Ishida T, Nishimura G, Katoh M, Miyazaki I.** Investigation of colonic prostaglandins in carcinogenesis in the rat colon. *Dis Colon Rectum* 1991; 34: 572–6.

44. **Pelus L, Strausser H.** Prostaglandins and the immune response. *Life Sci* 1977; 20: 903–14.
45. **Young M, Dizer M.** Enhancement of immune function and tumor growth inhibition by antibodies against prostaglandin E2. *Immunol Commun* 1983; 12: 11–23.
46. **Arvind P, Papavassiliou E, Tsioulias G, et al.** Prostaglandin E2 down-regulates the expression of HLA-DR antigen in human colon adenocarcinoma cell lines. *Biochemistry* 1995; 34: 5604–9.
47. **Qiao L, Kozoni V, Tsioulias GJ, et al.** Selected eicosanoids increase the proliferation rate of human colon carcinoma cell lines and mouse colonocytes *in vivo*. *Biochim Biophys Acta* 1995; 1258: 215–23.
48. **Uribe A, Alam M, Midtvedt T.** E2 prostaglandins modulate cell proliferation in the small intestinal epithelium of the rat. *Digestion* 1992; 52: 157–64.
49. **Pugh S, Thomas GAO.** Patients with adenomatous polyps and carcinomas have increased colonic mucosal prostaglandin E2. *Gut* 1994; 35: 675–8.
50. **Rao CV, Simi B, Reddy BS.** Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. *Carcinogenesis* 1993; 14: 2219–25.
51. **Rao CV, Desai D, Simi B, Kulkarni N, Amin S, Reddy BS.** Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant crypt foci formation in rat colon. *Cancer Res* 1993; 53: 4182–8.
52. **Boolbol S, Dannenberg A, Chadburn A, et al.** Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. *Cancer Res* 1996; 56: 2556–60.
53. **Rao CV, Simi B, Wynn T-T, Garr K, Reddy BS.** Modulating effect of amount and types of dietary fat on colonic mucosal phospholipase A2, phosphatidylinositol-specific phospholipase C activities, and cyclooxygenase metabolite formation during different stages of colon tumor promotion in male F344 rats. *Cancer Res* 1996; 56: 532–7.
54. **Sano H, Kawahito Y, Wilder RL, et al.** Expression of cyclooxygenase-1 and -2 in human colorectal cancer. *Cancer Res* 1995; 55: 3785–9.
55. **Kargman SL, O'Neill GP, Vickers PJ, Evans JF, Mancini JA, Jothy S.** Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res* 1995; 55: 2556–9.
56. **Kutchera W, Jones D, Matsunami N, et al.** Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: evidence for a transcriptional effect. *Proc Natl Acad Sci USA* 1996; 93: 4816–20.
57. **Gustafson-Svärd C, Lilja I, Hallböök O, Sjödahl R.** Cyclooxygenase-1 and cyclooxygenase-2 gene expression in human colorectal adenocarcinomas and in azoxymethane induced colonic tumours in rats. *Gut* 1996; 38: 79–84.
58. **DuBois RN, Radhika A, Reddy BS, Entigh AJ.** Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. *Gastroenterology* 1996; 110: 1259–62.
59. **Williams C, Luongo C, Radhika A, et al.** Elevated cyclooxygenase-2 levels in Min mouse adenomas. *Gastroenterology* 1996; 111: 1134–40.
60. **Reddy B, Rao C, Seibert K.** Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. *Cancer Res* 1996; 56: 4566–9.
61. **Takahashi M, Fukutake M, Yokota S, Ishida K, Wakabayashi K, Sugimura T.** Suppression of azoxymethane-induced aberrant crypt foci in rat colon by nimesulide, a selective inhibitor of cyclooxygenase 2. *J Cancer Res Clin Oncol* 1996; 122: 219–22.
62. **Oshima M, Dinchuk JE, Kargman SL, et al.** Suppression of intestinal polyposis in Apc^{716} knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 1996; 87: 803–9.
63. **Tsuji M, DuBois RN.** Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 1995; 83: 493–501.
64. **DuBois RN, Shao J, Tsujii M, Sheng H, Beauchamp RD.** G1 delay in cells overexpressing prostaglandin endoperoxide synthase-2. *Cancer Res* 1996; 56: 733–7.
65. **Plummer SM, Hall M, Faux SP.** Oxidation and genotoxicity of fecapentaene-12 are potentiated by prostaglandin H synthase. *Carcinogenesis* 1995; 16: 1023–8.
66. **Clària J, Lee M, Serhan C.** Aspirin-triggered lipoxins (15-epi-LX) are generated by the human lung adenocarcinoma cell line (A549)-neutrophil interactions and are potent inhibitors of cell proliferation. *Mol Med* 1996; 2: 583–96.
67. **Clària J, Serhan C.** Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell–leukocyte interactions. *Proc Natl Acad Sci USA* 1995; 92: 9475–9.
68. **Bozza P, Payne J, Morham S, Langenbach R, Smithies O, Weller P.** Leukocyte lipid body formation and eicosanoid generation: cyclooxygenase-independent inhibition by aspirin. *Proc Natl Acad Sci USA* 1996; 93: 11091–6.