Invited Review

Chemopreventive effects of NSAIDs against colorectal cancer: regulation of apoptosis and mitosis by COX-1 and COX-2

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Summary. There is a wealth of evidence that non-steroidal anti-inflammatory drugs (NSAIDs) can prevent colorectal cancer. In this article the role of cyclo-oxygenase 1 and 2, the principle target of NSAIDs, in the development of colorectal cancer is reviewed. Cyclooxygenase is constituitevely expressed in normal colonic epithelium and surrounding stroma and could catalyse the generation of malondialdehyde which is a known mutagen and could initiate colorectal carcino-genesis. Mutation of APC which is an early genetic event leads to the expression of cyclooxygenase 2 which may prevents the appropriate apoptosis of mutant adenoma cells. Other proneoplastic effects of cyclo-oxygenase include changing the action of Transforming Growth Factor ß from anti-proliferative to pro-proliferative, reducing adherence to extracellular matrix, promotes metastasis and angiogenesis. These properties of cyclooxygenases suggest that inhibition of both isoforms may have important protective effects against colorectal cancer.

Key words: Cyclooxygenase enzymes, Colorectal neoplasia, Apoptosis, Intestine

Introduction

There has been much interest in prevention of colorectal cancer in recent years. Current strategies depend on either dietary modification (Bingham, 1996), removal of adenomatous polyps (Winawer et al., 1997), detection of oncogene products in stool (Sidransky et al., 1992) or use of non-steroidal anti-inflammatory drugs (NSAIDs). In this article the rationale and mechanism of action of NSAIDs in the prevention of colorectal cancer will be reviewed paying particular attention to the role of cyclooxygenase isoenzymes, their principle therapeutic

target.

Evidence that NSAIDs prevent colorectal cancer

There are three lines of evidence that NSAIDs prevent colorectal cancer. First a number of animal studies have shown that a variety of NSAIDs, including sulindac (Rao et al., 1995), piroxicam (Reddy et al., 1987) and aspirin (Reddy et al., 1993) can prevent chemically-induced cancer in rodents. The degree of reduction in tumour burden in these studies was sometimes as high as 60% and was associated with a substantial reduction in the tissue concentration of eicosanoid products.

The second line of evidence is that NSAIDs can reduce the number and size of polyps in patients with familial adenomatous polyposis (FAP). FAP is an autosomal dominant condition resulting from mutations in the adenomatous polyposis coli gene which located on chromosome 5q21 which inevitably results in colorectal cancer by the age of 40 (Kinzler et al., 1991). The original observation by Waddell and colleagues in 1983 that sulindac reduced the number polyps in FAP patients has been confirmed by a number of studies including two double-blinded, randomised placebo-controlled trial of sulindac (Waddell and Loughry, 1983; Labayle et al., 1991; Giardiello et al., 1993).

However the most compelling comes from prospective cohort studies of the development of spontaneous colorectal cancer. In a cohort of over one million persons men who used more aspirin more than 16 times a month had a relative risk of developing colorectal cancer of 0.48 (0.30-0.76) and women a relative risk of 0.53 (0.32-0.87) (Thun et al., 1991). In a later prospective cohort prospective clinical studies of non-medical health care workers between ages 40 and 75 who regularly and consistently tool aspirin had a relative risk of 0.35 (0.16-0.75) (Giovannucci et al., 1994). An important aspect of this study was that prior use of endoscopic evaluation of the colon and other confounders were controlled for by multivariate analysis. In a further cohort study of 89,446

nurses Giovannucci and colleagues reported a reduced relative risk of developing colorectal cancer of 0.62 (0.44-0.86) (Giovannucci et al., 1995). Analysis of duration of use showed that the protective effect of aspirin did not become statistically apparent unless intake had been for 10 years or greater. This was attributed to the idea that adenomas take approximately 10 years to evolve into invasive carcinomas. In contrast to these large studies, two randomised controlled clinical trials of sulindac in patient with «sporadic» adenomatous polyps have failed to show any beneficial effect (Hixson et al., 1993; Landenheim et al., 1995). However these trials were small with inadequate statistical power and would not have been capable of detecting a response of less than a 50%. However, preliminary results from a randomised controlled trial of sulindac 150 mg twice daily for one year in patients with patients with 4-12 mm rectosigmoid polyps has shown a significant reduction in polyp size; the effect of sulindac on polyps number is not reported (DiSario et al., 1997).

Thus there is a range of independent data giving compelling evidence that NSAIDs prevent colorectal cancer. How does this occur? Before we attempt to answer this question we must first address the biology of cyclooxygenase enzymes which are the principle targets of NSAIDs.

Arachidonic acid metabolism

The therapeutic actions of NSAIDs are believed to be due to their effect on prostaglandin (eicosanoid) biosynthesis. It is therefore important to review their biochemistry. The first step in eicosanoid biosynthesis is liberation of arachidonic acid from membrane phospholipids by phospholipases. A number of phospholipases have been identified including one called secretory phospholipase A2 which is found in colonic mucosa and may be of particular importance in the development of colorectal cancer. In the mouse this enzyme is believed to be coded by Mom1 a gene locus which has been shown to modify the number of intestinal tumours produced in mice which develop a syndrome analagous to FAP as a result of mutation in APC. Mice expressing Mom1 have substantially fewer intestinal tumours than Mom1 null mice (Gould and Dove, 1996). The mechanism underlying the anti-tumour effects of phospholipase A2 are unknown but may involve modification of the lipid environment of intestinal epithelial cell.

Arachidonic acid is a 20 carbon unsaturated fatty acid which is metabolised via three main pathways. The first is the cyclooxygenase pathway in which arachidonic acid is converted to prostaglandin G/H_2 by prostaglandin G/H synthase-1 (PGHS-1) (8,11,14-eicosatrienoate, hydrogen donor: oxygen oxidoreductase, EC 1.14.99.1). This enzyme is referred to as cyclooxygenase-1 (COX-1) in this review. COX-1 has two different catalytic activities: it cyclizes and oxygenates arachidonic acid to form PGG_2 and also reduces PGG_2

to PGH_2 which is an alcohol. PGH_2 is then converted to PGE_2, $PGF_{2\alpha}$, $PGD_{2\alpha}$, PGI_2 and thromboxane A_2. There is little information on the effects of these compounds on colonic epithelial proliferation or death. PGE_2, $PGF_{2\alpha}$ and $PGI2$ can induce proliferation in human adenocarcinoma cell lines SW1116 and HT-29 (Qiao et al., 1995). PGE_2 and PGD2 can undergo further metabolism to dehydration products termed cyclopentone PGs. For example, dehydration of PGE_2 yields PGA_2 while dehydration of PGD_2 yields PGJ_2 and $\Delta12$-PGJ_2. The later can undergo a second dehydration step to 15-deoxy-$\Delta12$-PHJ_2. These cyclopentone PGs are of interest because they have potent antiproliferative activity in breast, lung and leukaemia cell lines (Fukushima et al., 1989), though they have the opposite effect in the breast cell line MCF-7 (Shahabi et al., 1987).

The second major metabolic pathway of arachidonic acid is via lipoxygenases which insert a single O_2 to form hydroperoxyeicoatetraenoic acids (HPETEs) which are then converted into hydroxyeicosatetraenoic acids (HETEs). Further metabolism then takes place to produce leukotrienes and lipoxins. Whether these arachidonic acid products play a role in carcinogenesis is unknown.

The final major metabolic pathway of arachidonic acid involves enzymes of the cytochrome P450 family which insert an atom of oxygen at various positions in the arachidonic acid molecule to produce 5-, 8-, 9,- 11-, 12- and 15-HETE (Levy, 1997).

Molecular biology of cyclooxygenase enzymes

COX-1 enzyme was first purified from bovine vesicular glands in 1976. COX-1 is expressed in most tissues at a relatively stable level and therefore is not considered a site for regulation of prostaglandin production. The COX-1 gene spans 22.5 kb and is composed of 11 exons and 10 introns and maps to chromosome 9q32-q33.3. Transcription of COX-1 yields a mRNA 2.7 kb in size which encodes a 565 residue, 65-kDa protein. This contains a short signal peptide to anchor it membranes and 4 possible N-linked glycosylation sites.

COX-2, also known as prostaglandin endoperoxide synthase-2 was identified in 1989 (Simmons et al., 1989; Kujubu et al., 1991). In contrast to COX-1, COX-2 expression can be induced by a wide variety of stimuli including; forskolin, interleukin-1, tumour necrosis factor, epidermal growth factor, transforming growth factor-α and retinoic acid. Under resting conditions COX-2 expression is virtually absent but is rapidly upregulated after stimulation with mitogens with consequent stimulation of PG production. The COX-2 gene is 8.3 kb in size. Most of the exons in COX-1 are preserved but the gene is smaller due to smaller introns. It is located on chromosome 1q25.2-q25.3. Transcription of COX-2 makes a mRNA of 4.5 kb in size which encodes a 70 kDa protein which has a 70% homology to

COX-1. There are slight differences between the two proteins thought the cyclooxygenase and peroxidase sites are preserved these differences could account for differences between the two enzymes. For example, aspirin inhibits COX-1 completely but only partially inhibits COX-2 allowing it to function as a 15-lipoxygenase converting arachidonic acid to 15-R-HETE. The 3-dimensonal structure of COX-2 has been determined at 3.0Å resolution. The overall structure of COX-1 and COX-2 are similar though COX-1 has a larger NSAID-binding site than COX-2. However there are sufficient differences to allow COX-2 to bind NSAIDs which are unable to bind to COX-1 (Kurumbail et al., 1996).

With the intestine both COX-1 and COX-2 are expressed in the submucosa but only COX-1 is expressed in the epithelial cells (unpublished observations) (Williams et al., 1996). Expression levels are similar in the small and large intestine. At a subcellular level both isoenzymes are found in the endoplasmic reticulum (Song and Smith, 1996). COX-2 is also found attached to the nuclear membrane.

Relationship between COX and genes involved in colorectal cancer

Currently there are two genetic events which are known to be involved in initiation of the colorectal neoplasia; truncation of the APC gene and mutation of mismatch repair genes (Grogan et al., 1996). Germ line mutations to both sets of genes give rise to hereditary colorectal cancer though the two syndromes have markedly different clinical features. Mutation of APC which almost always results in truncation of the protein gives rise to Familial polyposis coli in which affected individuals develop thousands of colonic adenomas during adolescence. One or more of these inevitably develop into an invasive fatal carcinoma in the fourth decade unless treated by colectomy. Crucially, similar truncation mutations also occur in about 85% of sporadic colorectal cancers and are again an early event. Roughly 15% of colorectal cancers demonstrate an inability to adequately repair DNA following certain types of DNA damage. This genetic abnormality can be identified as errors in stretches of dinucleotide or trinucleotide repeats found throughout the genome called DNA micro-satellites (Kinzler and Vogelstein, 1996). This genetic error is often referred to as replicative error phenotype (RER). Patients with Hereditary Non-Polyposis Colon Cancer (Lynch syndrome) have inherited mutations in one of five DNA mismatch repair genes which results in RER. Patients with RER demonstrate a far higher rate of mutation which results in faster progression of tumours from benign adenoma to invasive carcinoma than patients without RER. However they do not have the high number of adenomas found in FAP, nor do polyps develop in adolescence.

The epidemiological evidence reviewed above strongly suggests that COX in some way promotes the development of these crucial mutations. One of the products of PGH_2 transformation is malondialdehyde (MDA) which is mutagenic in bacteria and mammalian cells and carcinogenic in rodents (Mukai and Goldstein, 1976; Basu and Marnett, 1983; Spalding, 1988). MDA reacts with DNA to form the adduct M1guanine which can induce transitions to adenosine and transversions to thymidine (Moriya et al., 1994). This biochemistry raises the possibility that inhibition of submucosal COX-1 by NSAIDs could reduce the mutation frequency in colonic epithelium and prevent polyp initiation and could explain how NSAIDs such as aspirin or sulindac which have far greater potency against COX-1 than COX-1 inhibit polyp formation.

APC and COX-2

There appears to be a close relationship between APC mutation, COX-2 expression and the development of intestinal tumours. Mice with a mutation at amino acid number 716 develop hundreds of adenomas over a period of a few months (Oshima et al., 1996). Examination of these adenomas shows that unlike normal intestinal epithelial cells they express high levels of COX-2. APC causes upregulation of COX-2 expression however it is known that one of the functions of normal APC protein is to sequester ß-catenin protein. Mutation of APC usually results in truncation of APC protein, loss of ß-catenin binding and a rise in levels of free ß-catenin within the cytoplasm (Inomata et al., 1996). It has recently been shown that under these circumstances ß-catenin can translocate to the nucleus and bind to the transcription factor LEF-1 and thereby regulate gene expression possibly including COX-2 (Korinek et al., 1997a,b; Rubinfeld et al., 1997). When APCΔ716 mice are crossed with homozygous COX-2 null mice their progeny have seven-fold fewer intestinal tumours than APCΔ716 controls. Crossing APCΔ716 mice with which are COX-2 (+/-) mice results in an intermediate number of tumours. Furthermore treatment of APCΔ716/+ mice with an NSAID which inhibits COX-2 selectively also reduces tumour formation significantly. Together these data suggest that mutation of APC causes the upregulation of COX-2 whose products cause further tumour progression.

Role of apoptosis in colorectal cancer

COX products may act to prevent apoptosis and antagonism of this property may account for some of the anticancer actions of NSAIDs. A number of lines of evidence suggest that failure of appropriate apoptosis of cells with potentially malignant mutations play a significant role in the evolution of normal epithelium into a malignant tumour. In the small intestine apoptosis is targeted to the stem cells whereas in the colon the absolute levels of apoptosis are less and are not targeted away from the stem cells. This raises the possibility that the low incidence of small intestinal cancer relative to

colorectal cancer is due, in part, to colonic stem cells being resistant to apoptosis (Potten et al., 1992). Support for this idea comes from study of the effect of the carcinogens N-nitroso-N-methylurea, N-nitroso-N-ethylurea, 1,2 dimethylhydrazine and N-nitrosodimethyl-amine on apoptosis in the small and large intestine. In the small intestine all four carcinogens induced apoptosis at cell positions 4-6 suggesting that the DNA damage induced by these agents efficiently initiates apoptosis of stem cells. By contrast, in the colon apoptosis was maximally induced at cell positions 5 - 10 counting from the crypt base, avoiding the stem cells located at cells positions 1-2 (Li et al., 1992; Potten et al., 1992).

Further evidence for defective apoptosis playing a role in colorectal carcinogenesis comes from consideration of the function of genes which are mutant in colorectal cancer. Overexpression of APC in colorectal epithelial cells with mutant APC induces apoptosis (Morin et al., 1996).

Levels p53 of protein rise as a result of DNA damage to induces either G1 arrest or apoptosis and is mutant or deleted in 80% of cases of colorectal cancer (Baker et al., 1990). Radiation-induced DNA damage induces substantial apoptosis at the base of the crypts in p53 wild-type mice when examined 4.5 h later. This apoptosis is mediated by p53 as it completely blocked in p53 null transgenic mice. Furthermore p53 is expressed at the same cell positions in the small intestine as those which undergo apoptosis whereas in the colon the correlation is much less precise corroborating the idea that stem cells from the colon are much less susceptible to apoptosis than those from the small intestine. p53 has no influence on spontaneous apoptosis as this is unaltered in p53 null mice (Merritt et al., 1994). We have obtained similar results following administration 40mg/kg 5-FU (Pritchard et al., 1997).

Bcl-2, an anti-apoptotic protein, is expressed in colonic tumours with a higher proportion of adenomas than carcinomas being positive (Watson et al., 1996). In the normal intestine bcl-2 is expressed at cell positions 1 and 2 the colon where the stem cells are believed to be located. It is barely expressed in the small intestine. Experiments in bcl-2 null transgenic mice have shown that bcl-2 acts to suppress both spontaneous and radiation and 5-FU-induced apoptosis in the colon (Merritt et al., 1995; Pritchard et al., 1996). Of the other bcl-2 family members studied so far in colorectal neoplasia Bcl-x_L is over-expressed in 60% of carcinomas while the pro-apoptotic protein bak has reduced expression compared to normal tissue in 90% of carcinomas. There is little change in bax (Krajewska et al., 1997).

Overall these data support the idea that colonic stem cells are relatively resistant to apoptosis so that stem cells with potentially malignant mutations be escape deletion and go on to develop into neoplastic clones. Once polyp initiation has occurred the accumulation of mutations result in the tumour becoming more resistant to apoptosis. Direct evidence for this has been obtained by comparing apoptosis rates in cells isolated from carcinomas, adenomas and normal colonic epithelium and showing a gradient of apoptosis with most being found in normal tissue and least in carcinoma (Bedi et al., 1995).

The relationship between chemoprevention of colorectal cancer by NSAIDs and apoptosis

There is currently little information on the induction of apoptosis in normal intestinal epithelium by NSAIDs. This is of importance as it is not clear at what stages in evolution of colorectal cancer NSAIDs act. Clinical and animal studies suggest that NSAIDs can not only cause regression of pre-existing adenomas but also reduce the number of adenomas forming by presumably acting on normal or near normal stem cells. Apoptosis has been observed in biopsy specimens from patients NSAID-induced colitis (Lee, 1993) and a flow cytometric study of isolated rectal epithelial cells from FAP patients has shown increased apoptosis (Pasricha et al., 1995). However there is currently little information on the site of apoptosis along the crypt/villus axis and whether putative stem cells are involved. We have been studying the effect of sulindac sulfide (a COX-1 inhibitors), sulindac sulfone (an inactive structural control for sulindac sulfide) and SC-58625 (a selective COX-2 inhibitor) on apoptosis in the large intestine. We find that sulindac sulfide causes a 2-fold increase in apoptosis at the level of the stem cell (positions 1-4). Sulindac sulfone is without effect suggesting that the apoptosis is due to inhibition of COX-1. SC-58625 is also without effect (unpublished data). This is probable because very little COX-2 protein is expressed in normal colonic epithelium. A number of studies have shown that sulindac sulfide and aspirin can induce apoptosis in a number of adenoma (Elder et al., 1996) and carcinomas cell lines (Piazza et al., 1995; Shiff et al., 1995; Yoshikawa et al., 1995). The mechanism by which NSAIDs induce apoptosis is currently unclear. One attractive hypothesis is that they act by inhibiting COX-2 reducing the production of COX-2 products which can induce apoptosis (Watson and DuBois, 1997). In favour of this hypotheses rat intestinal cells over-expressing COX-2 are resistant to butyrate-induced apoptosis which can be restored by addition of sulindac sulfide (Tsujii and Dubois, 1995). Significantly, over-expression of COX-2 increased bcl-2 expression. Sulindac may also be able to reduce expression of COX-2 protein (Boobol et al., 1996). Sulindac has also been reported to both induce apoptosis and a G1/S block in HT-29 cells. The cell cycle block was associated with a rise in p21$^{waf/1}$ and a decrease in Rb and mutant p53 protein level but none of these changes correlated with apoptosis (Goldberg et al., 1996).

However not all data is compatible with NSAIDs inducing apoptosis via inhibition of COX-2. Sulindac sulfide and piroxicam can induce apoptosis in HCT-15

cells which do not possess COX-2 transcripts (Hanif et al., 1996). There is also evidence that other arachidonic acid metabolites such as PGE$_2$, LTB4, a lipoxygenase product and and 12 R-HETÉ, a P450 product can stimulate proliferation in HT-29 cells but without effect on apoptosis (Tang et al., 1993; Qiao et al., 1995; Bortuzzo et al., 1996; Korystov et al., 1996).

In summary although the majority of current data is compatible with the notion that inhibition of COX-2 induces apoptosis it remains to be proved that this is the mechanism of the chemopreventive effects of NSAIDs in vivo. Moreover it is not known whether inhibition of COX-2 induces apoptosis in stem cells or cells less likely to give rise to neoplasms higher up the villus. Nor has it been fully explored whether NSAIDs induce apoptosis via changing levels of bcl-2 or other family members or by a separate pathway.

Other pro-neoplastic actions of COX-2 products

Expression of COX-2 by malignant intestinal epithelial cells has a number of other effects not related to apoptosis which enhances their malignant potential. TGF-ß inhibits proliferation in normal intestinal epithelium but this anti-proliferative effect is frequently lost after malignant transformation (Barnard et al., 1989; Hoosein et al., 1989; Manning et al., 1991). This change in the action of TGF-ß from anti-proliferative to proproliferative may be due to interactions between COX-2 expression, and TGF-ß (Sheng et al., 1997). In addition, Tsujii and Dubois have shown that RIE-1 cells expressing COX-2 adhere to extracellular matrix better than cells without COX-2 activity (Tsujii and Dubois, 1995). Furthermore in preliminary studies, Tsujii and colleagues have shown that forced over-expression of COX-2 in Caco-2 cells results in induction of vascular endothelial tubular morphogenesis vascular endothelial cells implicating and role of COX-2 in angiogenesis and metastasis. These effects are inhibited by treatment with NSAIDs (Tsujii et al., 1997).

Conclusions

From the therapeutic point of view the major question concerning the relationship between prostaglandins and colorectal cancer is the relative contribution is of COX-1 and COX-2 to the transformation of normal colonic tissue into a metastatic fatal cancer. This is because the majority of currently available NSAIDs are predominately COX-1 inhibitors and cause significant morbidity and mortality as a result of peptic ulceration. New selective COX-2 inhibitors hold the promise of largely avoiding these adverse reactions, though they may provoke other undesirable adverse reactions. COX-1 would appear to be of importance in the initiation of colorectal carcinogenesis. It is constitiutively expressed, capable of generating carcinogens including malondialdehyde and is potently inhibited by the NSAIDs which have been demonstrated to prevent the formation of colorectal cancer in epidemiological and clinical studies. Currently it unclear whether COX-2 plays any role in these early stages. Once polyp initiation has occurred COX-2 is expressed in epithelial cells and acts to inhibit apoptosis and drive the growth of the tumour through interactions with growth factors such as TGF-ß. COX-2 also enhances angiogenesis and cell migration thereby aiding metastasis. As selective COX-2 inhibitors are only starting to be used in the clinical area there is no information on whether inhibition of COX-2 is of any clinical benefit in the treatment of colorectal cancer.

References

Baker S.J., Peisinger A.C., Jessup J.M., Paraskeva C., Markowitz S., Willson J.K.V., Hamilton S. and Vogelstein B. (1990). p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res. 50, 7717-7722.

Barnard J.A., Beauchamp R.D., Coffey R.J. and Moses H.L. (1989). Regulation of intestinal epithelial cell growth by transforming factor type beta. Proc. Natl. Acad. Sci. USA 86, 1578-82.

Basu A.K. and Marnett L.J. (1983). Unequivocal demonstration that malonidialdehyde is a mutagen. Carcingenesis 3, 331-333.

Bedi A., Pasricha P.J., Aktar A.J., Barber J.P., Bedi G.C., Giardello F.M., Zehnbauer B.A. and Hamilton S.R. (1995). Inhibition of apoptosis during development of colorectal cancer. Cancer Res. 50, 1811-1816.

Bingham S.A. (1996). Epidemiology and mechanisms relating diet to risk of colorectal cancer. Nutritional Res. Rev. 9, 197-239.

Boobol S.K., Dannenberg A.J., Chadburn A., Martucci C., Guo X.J., Ramonetti J.T., Abreu-Goris M., Newmark H.L., Lipkin M.L., DeCrosse J.J. and Bertagnolli M.M. (1996). Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res. 56, 2556-2560.

Bortuzzo C., Hanif R., Kashfi K., Staiano-Coico L., Shiff S.J. and Rigas B. (1996). The effect of leukotriences B and selected HETEs on the proliferation of colon cancer cells. Biochim. Biophys. Acta 1300, 240-246.

DiSario J.A., Alberts D.S., Tietze C.C., Khullar S.K., Bohman V.D., Larsen B.R., Hixson L.R., Samowitz W.S., Reading J.C., Buys S.S., Dawson R. and Burt R.W. (1997). Sulindac induces regression and prevents progression of sporadic colorectal adenomas. Gastroenterology 112, A555.

Elder D.J., Hague A., Hicks D.J. and Paraskeva C. (1996). Differential growth inhibition by the aspirin metabolite salicylate in human colorectal tumor cell lines: enhanced apotosis in carcinoma and in vitro-transformed adenoma relative to adenoa cell lines. Cancer Res. 56, 2273-2276.

Fukushima M., Kato T., Narumiya S., Mizushima Y., Sasaki H., Terashima Y. and Santoro M.G. (1989). Prostaglandin A and J: Antitumour and antiviral prostaglandins. Adv. Prostanglan. Thrombo. Leukotr. Res. 19, 415-418.

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. (1993). Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. New Engl. J. Med. 328, 1313-1316.

Giovannucci E., Rimm E.B., Stamper M.J., Colditz G.A., Ascherio A.

and Willett W.C. (1994). Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. Ann. Intern. Med. 121, 241-246.

Giovannucci E., Egan K.M., Hunter D.J., Stampfer M.J., Colditz G.A., Willett W.C. and Speizer F.E. (1995). Aspirin and the risk of colorectal cancer in women. New Engl. J. Med. 333, 609-614.

Goldberg Y., Nassif II, Pittas A., Tsai L.L., Dynlacht B.D., Rigas B. and Shiff S.J. (1996). The Antiproliferative effect of sulindac and sulindac sulfide on Ht- 29 colon-cancer cells - alterations in tumor-suppressor and cell cycle-regulatory proteins. Oncogene 12, 893-901.

Gould K.A. and Dove W.F. (1996). Action of Min and Mom1 on neoplasia in ectopic intestinal grafts. Cell Growth Differ. 7, 1361-1368.

Grogan L., Behan K.A. and Johnston P.G. (1996). Recent advances in the biology of colorectal cancer. Histol. Histopathol. 11, 807-820.

Hanif R., Pittas A., Feng Y., Koutsos M.I., Qiao L., Shiff S.J. and Rigas B. (1996). Effects of nonsteroiddal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-independent pathway. Biochem. Pharmacol. 52, 237-245.

Hixson L.J., Earnest D.L., Fennarty M.B. and Sampliner R.E. (1993). NSAID effect on sporadic colon polyps. Am. J. Gastroenterol. 88, 1652-1656.

Hoosein N.M., McKnight M.K., Levine A.E., Mulder K.M., Childress K.E., Brattain D.E. and Brattain M.G. (1989). Differential sensitivity of subclass of human colon carcinoma cell lines to the growth inhibitory effects of transforming growth factor-ß1. Exp. Cell Res. 181, 442-453.

Inomata M., Ochiai A., Akimoto S., Kitano S. and Hirohashi S. (1996). Alteration of beta-catenin expression in colonic epithelial cells of familial adenomatous polyposis patients. Cancer Res. 56, 2213-2217.

Kinzler K.W. and Vogelstein B. (1996). Lessons from hereditary colorectal cancer. Cell 87, 159-170.

Kinzler K.W., Nilbert M.C., Su L.K., Vogelstein B., Bryan T.M., Levy D.B., Smith K.J., Preisinger A.C., Hedge P., McKechnie D., Finniear R., Markham A., Groffen J., Boguski M.S., Altscul S.F., Horii A., Ando H., Miyoshi Y., Miki Y., Nishisho I. and Nakamura Y. (1991). Identification of FAP locus genes from chromosome 5q21. Science 253, 661-665.

Korinek V., Barker N., Morin P.J., van Wichen D., de Werger R., Kinzler K.W., Vogelstein B. and Clevers H. (1997a). Activation of beta-catenin-TCF signalling in colon cancer by mutations in beta-catenin or APC. Science 275, 1787-1790.

Korinek V., Barker N., Morin P.J., van Wichen D., de Werger R., Kinzler K.W., Vogelstein B. and Clevers H. (1997b). Constituitive trans-criptional activation by a ß-catenin-TCF complex in APC -/- colon carcinoma. Science 275, 1784-1787.

Korystov Y.N., Dobrovinskaya O.R., Shaposhnikova V.V. and Eidus L.K. (1996). Role of arachidonic acid metabolism in thymocyte apoptosis after irradiation. FEBS Lett. 388, 238-241.

Krajewska M., Moss S.F., Krajewski S., Song K., Holt P.R. and Reed J.C. (1997). Elevated expression of Bcl-x and reduced bak in primary colorectal adenocarcinomas. Cancer Res. 56, 2422-2427.

Kujubu D.A. Fletcher B.S., Varnum B.C., Lim R.W. and Herschman H.R. (1991). TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclo-oxygenase homologue. J. Biol. Chem. 266, 12866-12872.

Kurumbail R.G., Stevens A.N., Gierse J.K., McDonald J.J., Stegeman R.A., Pak J.Y., Gildehaus D., Miyashiro J.M., Penning T.D., Seibert K., Isakson P.C. and Stallings W.C. (1996). Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature 384, 644-648.

Labayle D., Fischer D., Vielh P., Drouhin F., Pariente A., Bories C., Duhamel O., Trousset M. and Attali P. (1991). Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology 101, 635-639.

Landenheim J., Garcia G., Titzer D., Herzenberg H., Lavori P., Edson R. and Omary M.B. (1995). Effect of sulindac on sporadic colonic polyps. Gastroenterology 108, 1083-1087.

Lee F.D. (1993). Importance of apoptosis in the histopathology of drug related lesions in the large intestine. J. Clin. Pathol. 46, 118-122.

Levy G.N. (1997). Prostaglandin H synthases, nonsteroidal anti-inflammatory drugs, and colon cancer. FASEB J. 11, 234-247.

Li Y.Q., Fam C.Y., Winton D.J. and Potten C.S. (1992). Target cells for the cytotoxic effects of carcinogens in the murine small intestine. Carcinogenesis 13, 361-368.

Manning A.M., Williams A.C., Game S.M. and Paraskeva C. (1991). Differential sensitivity of human colonic adenoma and carcinoma cells to transforming factor beta (TGF-beta): conversion of an adenoma cell line to a tumorigenic phenotype is accompanied by a reduced response to the inhibitory effects of TGF-beta. Oncogene 6, 1471-1476.

Merritt A.J., Potten C.S., Kemp C.J., Hickman J.A., Ballmain A., Lane D.P. and Hall P.A. (1994). The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53-deficient mice. Cancer Res. 54, 614-617.

Merritt A.J., Potten C.S., Watson A.J.M., Loh D.Y., Nakayama K. and Hickman J.A. (1995). Differential expression of Bcl-2 in intestinal epithelia - correlation with attenuation of apoptosis in colonic crypts and the incidence of colonic neoplasia. J. Cell Sci. 108, 2261-2271.

Morin P.J., Vogelstein B. and Kinzler K.W. (1996). Apoptosis and APC in colorectal tumorigenesis. Proc. Natl. Acad. Sci. USA 93, 7950-7954.

Moriya M., Zhang W., Johnson F. and Grollman A.P. (1994). Mutagenic potency of exocyclic DNA adducts: Marked differences between Escherichia coli and simian kidney cells. Proc. Natl. Acad. Sci. USA 91, 11899-11903.

Mukai F.H. and Goldstein B.D. (1976). Mutagenicity of malondiadehyde, a decomposition product of peroxidised polyunsaturated fatty acids. Science 191, 868-869.

Oshima M., Dinchuk J.E., Kargman S.L., Oshima H., Hancock B., Kmong E., Tryaskos J.M., Evans J.F. and Taketo M.M. (1996). Suppression of intestinal polyposis in APCΔ716 knockout mice by inhibition of prostaglandin endoperoxide synthase-2 (COX-2). Cell 87, 803-809.

Pasricha P.J., Bedi A., O'Conner K., Rashid A., Aktar A.J., Zahurak M., Paintadosi S., Hamilton S.R. and Giardello F. (1995). The effects of sulindac on colorectal proliferation and apoptosis in familial adenomatous polyposis. Gastroenterology 109, 994-998.

Piazza G.A., Rahm A.L.K., Krutzch M., Sperl G., Paranka N.S., Gross P.H., Brendel K., Burt R.W., Alberts D.S., Pamukcu R. and Ahnen D.J. (1995). Antineoplastic drugs sulindac sulfide and sulindac sulfone inhibit cell growth by inducing apoptosis. Cancer Res. 1995, 3110-3116.

Potten C.S., Li Y.Q., O'Conner P.J. and Winton D.J. (1992). A possible explanation for the differential cancer incidence in the intestine, based on distribution of the cytotoxic effects of carcinogens in the

murine large bowel. Carcinogenesis 13, 2305-2312.

Pritchard D.M., Watson A.J.M., Potten C.S. and Hickman J.A. (1996). Characterization of 5-fluorouracil-induced apoptosis in murine small and large-intestine - Studies in p53-null and bcl-2-null transgenic mice. Gastroenterology 110, A578.

Pritchard D.M., Watson A.J.M., Potten C.S., Lambert A.L. and Hickman J.A. (1997). Inhibition by uridine but not thymidine of p53-dependent intestinal apoptosis initiated by 5-fluorouracil: Evidence for the involvement of RNA perturbation. Proc. Natl. Acad. Sci. USA 94, 1795-1799.

Qiao L., Kozoni V., Tsioulias G.J., Koutsos M.I., Hanif R., Shiff S.J. and Rigas B. (1995). Selected eicosanoids increase the proliferation rate of human colon-carcinoma cell-lines and mouse colonocytes in-vivo. Biochim. Biophys. Acta-Lipids Lipid Metabol. 1258, 215-223.

Rao C.V., Rivenson A., Simi B., Zang E., Kelloff G., Steele V. and Reddy B.S. (1995). Chemoprevention of colon carcinogenesis by sulindac, anonsteroidal anti-inflammatory agent. Cancer Res. 55, 1464-1472.

Reddy B.S., Maruyama H. and Kelloff G. (1987). Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a non steroidal antiiflammatory drug, during different stages or rat colon tumor development. Cancer Res. 47, 5340-5346.

Reddy B.S., Rao C.V., Rivenson A. and Kelloff G. (1993). Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis. Carcinogenesis 14, 1493-1497.

Rubinfeld B., Robbins P., Elgamil M., Albert I., Porfiri E. and Polakis P. (1997). Stabilization of beta-catenin by genetic defects in melanoma cell lines. Science 275, 1790-1792.

Shahabi C.J., Chengini N. and Wittliff J.L. (1987). Alterations of MCF-7 human brest cancer cell after prostaglandins PGA1 and PGF2alpha treatment. Exp. Cell Biol. 55, 18-27.

Sheng H., Shao J., Hooton E.B., Tsujii M., DuBois R.N. and Beauchamp R.D. (1997). Cyclooxygenase-2 induction and transforming growth factor (TGF)-beta growth inhibition in rat intestinal epithelial cells. Cell Growth Differ. 8, 463-470.

Shiff S.J., Qiao L., Tsai L.L. and Rigas B. (1995). Sulindac sulfide, an aspirin-like compound, inhibits proliferation, causes cell-cycle quiescence, and induces apoptosis in Ht-29 colon adenocarcinoma cells. J. Clin. Invest. 96, 491-503.

Sidransky D., Tokino T., Hamilton S.R., Kinzler K.W., Levin B., Frost P. and Vogelstein B. (1992). Identification of ras oncogene mutations in the stool of patients with curable colorectal tumours. Science 256, 102-105.

Simmons D.L., Levy D.B., Yanoni Y. and Erison R.L. (1989). Identification of a phorbol ester-repressible v-scr-inducible gene. Proc. Natl. Acad. Sci. USA 86, 1178-1182.

Song I. and Smith W.L. (1996). C-terminal Ser/Pro-Thi-Leu tetra-peptides of prostaglandin endoperoxide H synthases-1 and -2 target the enzymes to endoplasmic reticulum. Arch. Biochem. Biophys. 334, 67-72.

Spalding J.W. (1988). Toxicology and carcinogenesis studies of maolodialdehyde sodium salt (3-hydroxy-2-propenal, sodium salt) in F344/N rats and B6C3F1 mice. NTP Technical Report 331, 5-13.

Tang D.G., Chen Y.Q. and Honn K.V. (1993). Arachidonate lipoxy-genases as essential regulators of cell survival and apoptosis. Proc. Natl. Acad. Sci. USA 93, 5241-5246.

Thun M.J., Namboodiri M.M. and Heath C.W.J. (1991). Aspirin use and the reduced risk of fatal colon cancer. N. Engl. J. Med. 325, 1593-1596.

Tsujii M. and Dubois R.N. (1995). Alterations in cellular adhesion and apoptosis in epithelial-cells overexpressing prostaglandin-endo-peroxide synthase 2 Cell 83, 493-501.

Tsujii M., Kawano S., Nagano K., Tsujii S., Sawaoka H. and DuBois R.N. (1997). Induction of vascular endothelial tubular morpho-genesis by Caco-2 cells programmed to over-express cyclo-oxygenase-2. Gastroenterology 112, A3610.

Waddell W.R. and Loughry R.W. (1983). Sulindac for polyposis coli. J. Surg. Oncol. 24, 83-87.

Watson A.J.M. and DuBois R.N. (1997). Lipid metabolism and APC: implications for colorectal cancer production. Lancet 349, 444-445.

Watson A.J.M., Merritt A.J., Jones L.S., Askew J.N., Anderson E., Becciolini A., Balzi M., Potten C.S. and Hickman J.A. (1996). Evidence for reciprocity of bcl-2 and p53 expression in human colorectal adenomas and carcinomas. Br. J. Cancer 73, 889-895.

Williams C.S., Luongo C., Radhika A., Zhang T., Lamps L.W., Nanney L.B., Beauchamp R.D. and Dubois R.N. (1996). Elevated cyclo-oxygenase-2 levels in min mouse adenomas. Gastroenterology 111, 1134-1140.

Winawer S.J., Fletcher R.H., Miller L., Godlee F., Stolar M.H., Mulrow C.D., Woolf S.H., Glick S.N., Ganaits T.G., Bond J.H., Rosen L., Zapka J.G., Olsen S.J., Giardello F.M., Sisk J.E., Van Antwerp R., Brown-Davis C., Marciniak D.A. and Mayer R.J. (1997). Colorectal cancer screening: clinical guidelines and rationale. Gastroenterology 112, 594-642.

Yoshikawa R., Ichii S., Hashimoto T., Furuyama J.-I. and Utsunomiya J. (1995). Effect of aspirin on the induction of apoptosis in adeno-carcinoma cell lines. Oncol. Rep. 2, 361-364.