

## Adjuvant drug therapy for cancer patients

### Table of Contents

- > Induction of cyclo-oxygenase-2 mRNA by prostaglandin E-2 in human prostatic carcinoma cells.
- > Inhibition of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase pathway induces p53-independent transcriptional regulation of p21(WAF1/CIP1) in human prostate carcinoma cells
- > Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer.
- > COX-2 and colon cancer
- > Chemopreventive effect of N-(2-cyclohexyloxy-4-nitrophenyl)methane sulfonamide (NS-398), a selective cyclooxygenase-2 inhibitor, in rat colon carcinogenesis induced by azoxymethane.
- > Nonsteroidal anti-inflammatory drugs, eicosanoids, and colorectal cancer prevention.
- > Lovastatin Augments Sulindac-Induced Apoptosis in Colon Cancer Cells and Potentiates Chemopreventive Effects of Sulindac.
- > ras oncogenes in human cancer: a review.
- > Lovastatin-induced proliferation inhibition and apoptosis in C6 glial cells.
- > Etodolac (Lodine) in the treatment of osteoarthritis: recent studies.
- > Double blind evaluation of the long-term effects of etodolac versus ibuprofen in patients with rheumatoid arthritis.
- > The relationship between cyclooxygenase-2 expression and colorectal cancer

#### Induction of cyclo-oxygenase-2 mRNA by prostaglandin E-2 in human prostatic carcinoma cells.

British Journal of Cancer 75 ( 8 ): p 1111-1118 1997

Tjandrawinata R R; Dahiya R; Hughes-Fulford M Lab. Cell Growth, Veterans Affairs Med. Cent., 4150 Clement St., San Francisco, CA 94121, USA

Abstract: Prostaglandins are synthesized from arachidonic acid by the enzyme cyclo-oxygenase. There are two isoforms of cyclooxygenases: COX-1 (a constitutive form) and COX-2 (an inducible form). COX-2 has recently been categorized as an immediate-early gene and is associated with cellular growth and differentiation. The purpose of this study was to investigate the effects of exogenous dimethylprostaglandin E-2 (dmPGE-2) on prostate cancer cell growth. Results of these experiments demonstrate that administration of dmPGE 2 to growing PC-3 cells significantly increased cellular proliferation (as measured by the cell number), total DNA content and endogenous PGE 2 concentration. DmPGE-2 also increased the steady-state mRNA levels of its own inducible synthesizing enzyme, COX-2, as well as cellular growth to levels similar to those seen with fetal calf serum and phorbol ester. The same results were observed in other human cancer cell types, such as the androgen-dependent

LNCaP cells, breast cancer MDA-MB-134 cells and human colorectal carcinoma DiFi cells. In PC-3 cells, the dmPGE-2 regulation of the COX-2 mRNA levels was both time dependent, with maximum stimulation seen 2 h after addition, and dose dependent on dmPGE-2 concentration, with maximum stimulation seen at 5  $\mu$ -g ml<sup>-1</sup>. The non-steroidal anti-inflammatory drug flurbiprofen (5  $\mu$ -M), in the presence of exogenous dmPG-2, inhibited the up-regulation of COX-2 mRNA and PC-3 cell growth. Taken together, these data suggest that PGE 2 has a specific role in the maintenance of human cancer cell growth and that the activation of COX-2 expression depends primarily upon newly synthesized PGE-2, perhaps resulting from changes in local cellular PGE-2 concentrations.

### **Inhibition of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase pathway induces p53-independent transcriptional regulation of p21(WAF1/CIP1) in human prostate carcinoma cells**

**Journal of Biological Chemistry ( United States ) 24 APR 1998 , 273/17**

**Lee S.J.; Ha M.J.; Lee J.; Nguyen P.; Choi Y.H.; Pirnia F.; Kang W.-K.; Wang X.-F.; Kim S.-J.; Trepel J.B. J.B. Trepel, Medicine Branch, NCI, National Institutes of Health, Bethesda, MD 20892 United States trepel@helix.nih.gov**

Progression through the cell cycle is controlled by the induction of cyclins and the activation of cognate cyclin-dependent kinases. The 3- hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor lovastatin induces growth arrest and cell death in certain cancer cell types. We have pursued the mechanism of growth arrest in PC-3-M cells, a p53-null human prostate carcinoma cell line. Lovastatin treatment increased protein and mRNA levels of the cyclin-dependent kinase inhibitor p21(WAF1/CIP1), increased binding of p21 with Cdk2, markedly inhibited cyclin E- and Cdk2-associated phosphorylation of histone H1 or GST-retinoblastoma protein, enhanced binding of the retinoblastoma protein to the transcription factor E2F-1 in vivo, and induced the activation of a p21 promoter reporter construct. By using p21 promoter deletion constructs, the lovastatin-responsive element was mapped to a region between -93 and -64 relative to the transcription start site. Promoter mutation analysis indicated that the lovastatin-responsive site coincided with the previously identified transforming growth factor-beta- responsive element. These data indicate that in human prostate carcinoma cells an inhibitor of the HMG-CoA reductase pathway can circumvent the loss of wild-type p53 function and induce critical downstream regulatory events leading to transcriptional activation of p21.

### **Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer.**

**Cancer Res 1999 Mar 1;59(5):987-90**

**Tucker ON, Dannenberg AJ, Yang EK, Zhang F, Teng L, Daly JM, Soslow RA, Masferrer JL, Woerner BM, Koki AT, Fahey TJ 3rd  
Department of Surgery, New York Presbyterian Hospital and Weill Medical College of Cornell University, New York, New York 10021, USA.**

A large body of evidence suggests that cyclooxygenase-2 (COX-2) is important in gastrointestinal cancer. The purpose of this study was to determine whether COX-2 was expressed in adenocarcinoma of the human pancreas. Quantitative reverse transcription-PCR, immunoblotting, and immunohistochemistry were used to assess the expression of COX-2 in pancreatic tissue. Levels of COX-2 mRNA were increased by >60-fold in pancreatic cancer compared to adjacent nontumorous tissue. COX-2 protein was present in 9 of 10 cases of adenocarcinoma of the pancreas but was undetectable in nontumorous pancreatic tissue. Immunohistochemical analysis showed that COX-2 was expressed in malignant epithelial cells. In cultured human pancreatic cancer cells, levels of COX-2 mRNA and protein were induced by treatment with tumor-promoting phorbol esters. Taken together, these results suggest that COX-2 may be a target for the prevention or treatment of pancreatic cancer.

### **COX-2 and colon cancer**

**Taketo MM**

**Laboratory of Biomedical Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan.  
taketo@mol.f.u-tokyo.ac.jp**

The role of cyclooxygenase-2 (COX-2) in colorectal tumorigenesis in mice was studied by Oshima et al. to determine the effects of COX-2 gene knockouts and a new COX-2 inhibitor. In the study, heterozygous Apcdelta716 knockout mice, a mouse model of human familial adenomatous polyposis (FAP), were either crossed to COX-2 gene knockout mice, or fed chow containing the COX-

2-selective inhibitor. Apcdelta716 litter mates were used as positive controls, which developed 652 $\pm$ 198 (SD) polyps at 10 weeks. Introduction of a COX-2 gene mutation, or feeding with the COX-2-selective inhibitor to the Apcdelta716 knockout mice, reduced the number and size of intestinal polyps dramatically. The results provide direct genetic evidence that COX-2 plays a key role in tumorigenesis, and indicate that COX-2-selective inhibitors can be a new class of therapeutic agents for colorectal polyposis and cancer.

**Chemopreventive effect of N-(2-cyclohexyloxy-4-nitrophenyl)methane sulfonamide (NS-398), a selective cyclooxygenase-2 inhibitor, in rat colon carcinogenesis induced by azoxymethane.**

**Japanese J Cancer Res 1999 Apr;90(4):406-12**

**Yoshimi N, Shimizu M, Matsunaga K, Yamada Y, Fujii K, Hara A, Mori H Department of Pathology, Gifu University School of Medicine. yoshimi@cc.gifu-u.ac.jp**

Non-steroidal anti-inflammatory drugs (NSAIDs) such as sulindac and indomethacin inhibit colon carcinogenesis, and selective cyclooxygenase (COX)-2 inhibitors are considered to be potential chemopreventive agents without the side effects of usual NSAIDs. We reported that NS-398, N-(2-cyclohexyloxy-4-nitrophenyl)methane sulfonamide, suppressed the formation of preneoplastic lesions, aberrant crypt foci (ACF), induced by azoxymethane (AOM) in a short-term assay of rat colon carcinogenesis. In this study, we examined the effects of long-term NS-398 administration on rat colon carcinogenesis. After three AOM treatments at weekly intervals, a dose of 10 mg/kg of NS-398 in 5% Arabic gum solution was administered by gavage three times per week in group 2 until the termination of the experiment. Rats in group 1 were fed in a basal diet and given 5% Arabic gum solution alone after AOM treatment. At 40 weeks after the first AOM treatment, all rats were killed and the whole intestines including colon were examined. While the incidences of whole intestinal and colon neoplasms in group 1 were 84.6% and 80.8%, respectively, those in group 2 (given NS-398) were 51.9% and 44.4% respectively ( $P=0.0177$  and  $P=0.0103$  by Fisher's exact test, respectively). The multiplicities in group 2 ( $0.67\pm 0.78$  and  $0.48\pm 0.58$ ) were also decreased significantly compared with those ( $1.39\pm 1.10$  and  $1.08\pm 0.74$ ) in group 1 ( $P<0.01$  by Welch's method and  $P<0.002$  by Student's t test, respectively). In immunohistochemistry for proliferative cell nuclear antigen (PCNA), the PCNA-stained cell index ( $7.40\pm 0.5$ ) in group 2 was significantly decreased from that in group 1 ( $14.03\pm 0.82$ ) ( $P<0.001$  by Welch's method). The results suggest that NS-398, a selective COX inhibitor, has a chemopreventive activity against colon carcinogenesis without side-effects such as gastric ulceration.

**Nonsteroidal anti-inflammatory drugs, eicosanoids, and colorectal cancer prevention.**

**Gastroenterol Clin North Am 1996 Dec;25(4):773-91**

**DuBois RN, Giardiello FM, Smalley WE Department of Medicine, Veterans Affairs Medical Center, Nashville, Tennessee, USA.**

A concise review of the literature that evaluates the risk of colorectal cancer among NSAID users has been presented. Animal studies document a protective effect of NSAIDs in preventing colorectal cancers in carcinogen-induced (AOM) models and in Min mice. NSAIDs are protective in the animal model, even if given 14 weeks after administration of the carcinogen, indicating that these agents must be acting early in the adenoma-to-carcinoma sequence. Treatment of FAP patients with NSAIDs causes regression of adenomas that were already present before initiation of therapy. Many epidemiologic studies have examined the relationship between aspirin use and colorectal cancer. Most show a marked decrease in the relative risk (40% to 50%) of this tumor among continuous aspirin users. The appropriate dose and duration of aspirin treatment needed for optimal results are still unknown. Future work, directed at the molecular basis for the chemoprotective effects of NSAIDs in humans, may reveal strategies for the development of better chemopreventive agents. One effect shared by all NSAIDs is inhibition of cyclooxygenase. Presently, whether inhibition of COX-1 or COX-2 is required for the protective effect of aspirin and other NSAIDs is unclear. The authors and others have demonstrated that COX-2 is up-regulated from 2 to 50 fold in 85% to 90% of colorectal adenocarcinomas, making the COX-2 enzyme a more likely target. The authors have also reported a dramatic increase in COX-2 expression in colon tumors that develop in rats after AOM treatment. Drugs are currently being developed that preferentially inhibit either COX-1 or COX-2. If COX-2 is found to be a relevant target in the prevention of colorectal cancer, these newly developed, selective NSAIDs may play a role in future chemoprevention strategies.

**Lovastatin Augments Sulindac-Induced Apoptosis in Colon Cancer Cells and Potentiates Chemopreventive Effects of Sulindac.**

**Agarwal B, Rao CV, Bhendwal S, Ramey WR, Shirin H, Reddy BS, Holt PR Division of Gastroenterology, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York.**

**Background & Aims:** 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (HRI) were found incidentally to reduce new cases of colon cancer in 2 large clinical trials evaluating coronary events, although most patients in both treatment and control group were taking nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are associated with reduced colon cancer incidence, predominantly by increasing apoptosis. We showed previously that lovastatin induces apoptosis in colon cancer cells. In the present study we evaluated the potential of combining lovastatin with sulindac for colon cancer chemoprevention. **Results:** Lovastatin, 10-30  $\mu\text{mol/L}$ , augmented sulindac-induced apoptosis up to 5-fold in 3 colon cancer cell lines. This was prevented by mevalonate (100  $\mu\text{mol/L}$ ) or geranylgeranylpyrophosphate (10  $\mu\text{mol/L}$ ) but not farnesylpyrophosphate (100  $\mu\text{mol/L}$ ), suggesting inhibition of geranylgeranylation of target protein(s) as the predominant mechanism. In an azoxymethane rat model of chemical-induced carcinogenesis, the total number of colonic aberrant crypt foci per animal (control, 161  $\pm$  11) and the number of foci with 4+ crypts (control, 40  $\pm$  4.5) decreased to 142  $\pm$  14 (NS) and 43  $\pm$  2.9 (NS), respectively, with 50 ppm lovastatin alone; to 137  $\pm$  5.4 ( $P = 0.053$ ) and 36  $\pm$  2.1 (NS) with 80 ppm sulindac alone; and to 116  $\pm$  8.1 ( $P = 0.004$ ) and 28  $\pm$  3.4 ( $P = 0.02$ ) when 50 ppm lovastatin and 80 ppm sulindac were combined. **Conclusions:** Addition of an HRI such as lovastatin may augment chemopreventive effects of NSAIDs or/and may allow lower, less toxic doses of these drugs to be used.

### **ras oncogenes in human cancer: a review. Bos JL**

Published erratum appears in Cancer Res 1990 Feb 15;50(4):1352

Laboratory for Molecular Carcinogenesis, Sylvius Laboratory, Leiden, The Netherlands.

Mutations in codon 12, 13, or 61 of one of the three ras genes, H-ras, K-ras, and N-ras, convert these genes into active oncogenes. Rapid assays for the detection of these point mutations have been developed recently and used to investigate the role mutated ras genes play in the pathogenesis of human tumors. It appeared that ras gene mutations can be found in a variety of tumor types, although the incidence varies greatly. The highest incidences are found in adenocarcinomas of the pancreas (90%), the colon (50%), and the lung (30%); in thyroid tumors (50%); and in myeloid leukemia (30%). For some tumor types a relationship may exist between the presence of a ras mutation and clinical or histopathological features of the tumor. There is some evidence that environmental agents may be involved in the induction of the mutations.

### **Lovastatin-induced proliferation inhibition and apoptosis in C6 glial cells.**

**J Pharmacol Exp Ther 1999 Apr;289(1):572-9 Choi JW, Jung SE**

**Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea. jwchoiphar@yumc.yonsei.ac.kr**

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme in cholesterol biosynthesis. HMG-CoA reductase converts HMG-CoA to mevalonate, which is then converted into cholesterol or various isoprenoids through multiple enzymatic steps. In this study, we examined the cytotoxic effects of lovastatin, an HMG-CoA reductase inhibitor, in C6 glial cells. Lovastatin at concentrations higher than 10  $\mu\text{M}$  suppressed cell proliferation and induced cell death, which were prevented completely by mevalonate (300  $\mu\text{M}$ ). The data from lactate dehydrogenase assay and fluorescence microscopic assay using Hoechst 33342 and propidium iodide showed that mevalonate at a concentration of 100  $\mu\text{M}$  could prevent lovastatin-induced cell death, whereas it could not prevent lovastatin-induced inhibition of cell proliferation. These data suggest that the lovastatin-induced interruption of cell cycle transition was not sufficient to induce cell death in C6 glial cells. In the presence of lovastatin at concentrations higher than 10  $\mu\text{M}$ , DNA laddering, the typical finding of apoptosis, was identified. Lovastatin-induced apoptosis was prevented by mevalonate (100  $\mu\text{M}$ ). Both cycloheximide (0.5  $\mu\text{g/ml}$ ) and actinomycin D (0.1  $\mu\text{g/ml}$ ) prevented lovastatin-induced DNA laddering. In this study, we demonstrated that the cytotoxic effects of lovastatin fall into two categories: suppression of cell growth and induction of apoptosis in C6 glial cells.

### **Etodolac (Lodine) in the treatment of osteoarthritis: recent studies.**

**J Rheumatol Suppl 1997 Feb;47:23-31**

**Schnitzer TJ, Constantine G Rush Medical College, Department of Internal Medicine, Chicago, IL, USA.**

Etodolac (Lodine) has been marketed in the United States since 1991 for managing pain and for acute and longterm treatment of the signs and symptoms of osteoarthritis (OA). Etodolac was recently approved for the treatment of rheumatoid arthritis. We review the results of 3 recent 4 week, multicenter, placebo controlled, parallel group studies that compared the efficacy and safety of etodolac with naproxen and nabumetone. Because studies of etodolac in the treatment of OA concentrated on bid doses, the first study compared etodolac 800 mg/day given as 400 mg bid (106 patients) and 200 mg qid (105 patients) with naproxen 1000 mg/day (109 patients) and placebo (104 patients). Etodolac was as effective as naproxen, and the 2 dosage schedules of etodolac were comparable. The 2nd study compared etodolac 400 mg bid (86 patients) with naproxen 500 bid (82 patients) and placebo (86 patients). Etodolac was again found to be as effective as naproxen. The 3rd study compared etodolac 400 mg bid (91 patients) with nabumetone 1500 mg/day (89 patients) and placebo (90 patients). The results indicated that the efficacy of etodolac was comparable to that of nabumetone and resulted in significantly better scores at endpoint on the investigator's overall assessment and patient's global assessment. In all 3 studies there were no significant differences among the groups in the frequency of study events or premature discontinuations as a result of study events. The most common adverse event was digestive system disturbance, which was mild to moderate in severity. The results of these studies confirm the efficacy and safety of etodolac in managing the signs and symptoms of OA.

**Double blind evaluation of the long-term effects of etodolac versus ibuprofen in patients with rheumatoid arthritis.**

**J Rheumatol Suppl 1997 Feb;47:17-22**

**Neustadt DH Department of Medicine, University of Louisville School of Medicine, KY 40202, USA.**

We compared the longterm efficacy and safety of 2 dosages of etodolac with that of ibuprofen in the treatment of active rheumatoid arthritis (RA). The ability of etodolac to retard, arrest, reverse, or heal joint damage due to RA was also evaluated. Patients in the early stages of RA were assigned randomly to 3 parallel groups for up to 3 years of therapy: etodolac at 150 mg bid, etodolac at 500 mg bid, and ibuprofen 600 mg qid. Concurrent disease modifying antirheumatic drugs were not permitted; established low dosage corticosteroid therapy could be continued. A total of 1446 patients was enrolled. About 50% of patients completed one year; dropout rates were comparable between groups. Both etodolac dosages provided comparable efficacy to that of ibuprofen during the first 2 months; longterm assessment showed that 1000 mg/day of etodolac produced superior improvement as assessed by patients' opinions and number of swollen joints. About 2% of patients in each group achieved remission, and radiographs showed no difference in disease progression between treatments. The incidences of adverse events were comparable, although dyspepsia and rash occurred less frequently with 300 mg/day of etodolac than with 2400 mg/day ibuprofen. A higher incidence of gastrointestinal ulcers and bleeding was seen with ibuprofen. Changes in hepatic and renal function were of minor clinical significance and were similar between the 3 groups. Both dosages of etodolac were comparable to 2400 mg/day ibuprofen in treating RA. All 3 treatment regimens were well tolerated.

**The relationship between cyclooxygenase-2 expression and colorectal cancer**

**JAMA 1999 Oct 6;282(13):1254-7**

**Sheehan KM, Sheahan K, O'Donoghue DP, MacSweeney F, Conroy RM, Fitzgerald DJ, Murray FE Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin. ksheehan@rcsi.ie**

CONTEXT: Epidemiological studies have implicated the inducible form of cyclooxygenase (COX-2) in the pathogenesis of colorectal cancer; however, its role is not fully understood. OBJECTIVE: To examine the relationship between the expression of COX-2 in human colorectal cancer and patient survival. DESIGN: Patients diagnosed as having colorectal cancer were evaluated and followed up for up to 9.4 years (median follow-up, 2.7 years). Tumor sections were stained for COX-2 using a rabbit polyclonal antibody raised against human COX-2. The extent of COX-2 staining was graded by 2 observers blinded to outcome. Preabsorption of the anti-COX-2 antibody with a COX-2 peptide abolished the staining, demonstrating the specificity of the assay. SETTING: Gastrointestinal unit of a large general teaching hospital in Dublin, Ireland. PARTICIPANTS: Seventy-six patients (median age, 66.5 years) with colorectal cancer (Dukes tumor stage A, n = 9; Dukes B, n = 30; Dukes C, n = 25; Dukes D, n = 12) whose diagnosis was made between 1988 and 1991. Fourteen normal colon biopsies were stained for COX-2 as controls. MAIN OUTCOME MEASURES: Survival in years following diagnosis compared by extent of COX-2 epithelial staining (grade 1, <1%; grade 2, 1%-19%; grade 3, 20%-49%; grade 4, > or = 50%), Dukes stage, tumor size, and lymph node metastasis. RESULTS: COX-2 was found in tumor epithelial cells, inflammatory cells, vascular endothelium, and/or fibroblasts. The extent of epithelial staining was heterogeneous, varying markedly among different tumors. Normal tissue adjacent to the tumors also stained weakly for COX-2. No

COX-2 was detected in control tissue samples. The Kaplan-Meier survival estimate was 68% in patients who had grade 1 tumor epithelial staining compared with 35% in those with higher grades combined (log-rank  $\chi^2 = 5.7$ ;  $P = .02$ ). Greater expression of COX-2 correlated with more advanced Dukes stage (Kendall tau-b, 0.22;  $P = .03$ ) and larger tumor size (Kendall tau-b, 0.21;  $P = .02$ ) and was particularly evident in tumors with lymph node involvement (Kendall tau-b, 0.26;  $P = .02$ ). CONCLUSIONS: Our data indicate that COX-2 expression in colorectal cancer may be related to survival. These data add to the growing epidemiological and experimental evidence that COX-2 may play a role in colorectal tumorigenesis.

All Contents Copyright © 1995-2009 Life Extension Foundation All rights reserved.

**LifeExtension**<sup>®</sup>

These statements have not been evaluated by the FDA. These products are not intended to diagnose, treat, cure or prevent any disease. The information provided on this site is for informational purposes only and is not intended as a substitute for advice from your physician or other health care professional or any information contained on or in any product label or packaging. You should not use the information on this site for diagnosis or treatment of any health problem or for prescription of any medication or other treatment. You should consult with a healthcare professional before starting any diet, exercise or supplementation program, before taking any medication, or if you have or suspect you might have a health problem. You should not stop taking any medication without first consulting your physician.