

## Chapter 2

# The Role of Inflammation in Colon Cancer

Naveena B. Janakiram and Chinthalapally V. Rao

**Abstract** Colorectal cancer (CRC) is the one of the leading causes of cancer-related deaths in the world. CRC is responsible for more than 600,000 deaths annually and incidence rates are increasing in most of the developing countries. Epidemiological and laboratory investigations suggest that environmental factors such as western style dietary habits, tobacco-smoking, and lack of physical activities are considered as risks for CRC. Molecular pathobiology of CRC implicates pro-inflammatory conditions to promote the tumor malignant progression, invasion, and metastasis. It is well known that patients with inflammatory bowel disease are at higher risk of CRC. Many evidences exist reiterating the link between Inflammation and CRC. Inflammation involves interaction between various immune cells, inflammatory cells, chemokines, cytokines, and pro-inflammatory mediators, such as cyclooxygenase (COX) and lipoxygenase (LOX) pathways, which may lead to signaling towards, tumor cell proliferation, growth, and invasion. Thus, this review will focus on mechanisms by which pro-inflammatory mediators and reactive oxygen/nitrogen species play a role in promoting CRC. Based on these mechanisms, various preventive strategies, involving anti-inflammatory agents, such as COX inhibitors, COX-LOX inhibitors, iNOS inhibitors, natural supplements/agents, and synthetic agents, that blocks the inflammatory pathways and suppress CRC are discussed in this review.

---

N. B. Janakiram (✉) · C. V. Rao (✉)

Center for Cancer Prevention and Drug Development, Department of Medicine, Hematology Oncology Section, PCS Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA  
e-mail: naveena-janakiram@ouhsc.edu

C. V. Rao  
e-mail: cv-rao@ouhsc.edu

## Abbreviations

CRC	Colorectal cancer
CAC	Colitis-associated cancer
IBD	Inflammatory bowel disease
NSAIDs	Non-steroidal anti-inflammatory drugs
NK	Natural killer cells
DC	Dendritic cells
ACF	Aberrant crypt foci
T reg	T regulatory cells
5-ASA	5-aminosalicylic acid
MCP-1	Monocytes chemo attractant protein 1
PGE <sub>2</sub>	Prostaglandin E <sub>2</sub>
IL-8	Interleukin-8
IL-6	Interleukin-6
IL-10	Interleukin 10
TNF- $\alpha$	Tumor necrosis factor- $\alpha$
COX-2	Cyclooxygenase-2
PGI <sub>2</sub>	Prostaglandin I <sub>2</sub>
PGD <sub>2</sub>	Prostaglandin D <sub>2</sub>
LT	Leukotriene
HPETE	Hydroperoxyeicosatetraenoic acid
EETs	Epoxy-eicosatrienoic acids
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
PUFAs	Polyunsaturated fatty acids (PUFAs)
LX	Lipoxins
RVs	Resolvins
AOM	Azoxymethane
NO	Nitric oxide
NF- $\kappa$ B	Nuclear factor- $\kappa$ B
MMP	Matrix metalloproteinase
PI3K	Phosphatidylinositol 3-kinase
mPGES	Microsomal prostaglandin E synthase
VEGF	Vascular endothelial growth factor
FLAP	Five lox activating protein
DP1	PGD <sub>2</sub> receptor
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
NO	Nitric oxide
iNOS	Inducible nitric oxide synthase
nNOS	Neuronal nitric oxide synthase
eNOS	Endothelial nitric oxide synthase
APC	Adenomatous polyposis coli
LPS	Lipopolysaccharide

IL-1 $\beta$	Interleukin-1 $\beta$
AA	Arachidonic acid
NO-NSAID	NO-releasing NSAID
IL-4	Interleukin 4
COXibs	COX-2-specific inhibitors
FAP	Familial adenomatous polyposis
L-NAME	<i>L</i> -nitro arginine methyl ester
Se-PBIT	Selenium [ <i>S,S'</i> -1,4-phenylenebis(1,2-ethanediyl) bis-isothiourea]
GI	Gastrointestinal
MIP	Macrophage inflammatory protein
MCP	Monocytes chemo attractant protein
ABC	ATP-binding cassette
DMH	Dimethyl hydrazine
MDFs	Mucin depleted foci
DSS	Dextran sulfate sodium
EPA-FFA	Eicosapentaenoic acid-free fatty acid
ONA	Oleanolic acid
OT	18 $\alpha$ -olean-12-ene-3 $\beta$ -23,28-triol
18 <i>R</i> -RvE1	5,12,18 <i>R</i> -trihydroxy-EPA
LXA <sub>4</sub>	lipoxins A <sub>4</sub>
ABC	ATP-binding cassette

## 2.1 Colorectal Cancer: A Major Health Problem

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in the United States (US). Early diagnosis, though, often can lead to a complete cure. Each year, worldwide more than 1.2 million cases are diagnosed with about 600,000 deaths. CRC is the third most common cancer diagnosed in both men and women in the US and the fourth most common cause of cancer mortality worldwide (Tenesa and Dunlop 2009). It is the second most common cause of cancer deaths in the US. Overall, the lifetime risk of developing CRC is ~6%. As per American Cancer Society statistics, it is expected to cause about 50,830 deaths during 2013 (ACS). Most of the CRC cases are sporadic and about 25% are linked to genetic disorders. The majority of CRC cases are linked to environmental factors, including diet, exercise, weight, food borne mutagens, intestinal commensals, and chronic intestinal inflammation, which precedes tumor development.

## 2.2 Inflammatory Bowel Disease as Risk Factor for Colorectal Cancer

Inflammatory bowel disease (IBD) may lead to colitis-associated cancer (CAC), which is a usually difficult-to-treat cancer having high mortality (Feagins et al. 2009). It is reported that more than 20% of IBD patients develop cancer and 50% of these will

die of colon cancer (Lakatos and Lakotos 2008). These patients are reported to have increased inflammatory infiltration and increased expression of inflammatory genes (Atreya and Neurath 2008; Atreya et al. 2008; Waldner and Neurath 2008; Clevers 2004). A higher risk for colon cancer is observed in IBD patients who have a family history of CRC (Askling et al. 2001). This increased risk suggests an overlap of signaling pathways and mechanisms that drive cancer development in CAC and CRC. Anti-inflammatory therapy has been reported to reduce the risk or prevent CRC and colitis-related CRC in several observational studies. Non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to reduce colorectal neoplasia by 40–50 % (Thun et al. 1991; Smalley and Dubois 1997) and also have been reported to reduce CRC mortality odds by 49 % in a population of US military veterans with IBD (Bansal and Sonnenberg 1996). A recent meta-analysis of 9 observational studies reported that use of 5-aminosalicylic acid (5-ASA), mesalamine reduced the odds of colitis-related CRC by 49 % (Velayos et al. 2006). It is noteworthy that anti-inflammatory drugs such as 5-aminosalicylate-based compounds have remained in the mainstream for the treatment of IBD patients for >50 years. The findings in the human studies confirm observations in animal models, which show that NSAIDs reduce the occurrence of intestinal neoplasia. More than 90 % of 110 preclinical animal studies examining the effects of NSAIDs on tumorigenesis reported an anti-neoplastic effect (Hawk and Levin 2005). The large volume of compelling data on the use of anti-inflammatory agents/NSAIDs to reduce the risk of CRC suggests a potential role of inflammation in CRC.

### 2.3 Inflammation in CRC

Inflammation is driven by the accumulation of various immune and inflammatory cells and soluble inflammatory mediators, such as cytokines, chemokines, growth factors, lipid molecules, reactive oxygen, and nitrogen species. The interaction between these immune and inflammatory cells and cytokines leads to generation of autocrine and paracrine signals that foster tumor cell progression, growth, and metastases. A clear link exists between inflammation and CRC. Even CRC that is linked to genetic mutations shows a contribution from inflammation to tumor development, as shown by the decreased CRC mortality with regular use of NSAIDs. These data strongly support a pro-tumorigenic role of inflammation in colon cancer. Various factors can influence the initiation of inflammation and establishment of CRC.

### 2.4 Role of Immune and Inflammatory Cells in CRC

Pathological analysis of CRC shows infiltration with various types of cells that function in innate immunity, such as neutrophils, mast cells, natural killer (NK) cells, dendritic cells (DC), and tumor-associated macrophages (Atreya and Neurath 2008).

These cells help in anti-tumor immune responses by suppressing tumor growth and angiogenesis. They also help to recruit and interact with other cells involved in adaptive immune responses. Collectively, these responses lead to a balancing of immune surveillance with tumor-promoting inflammatory functions. Immune surveillance helps in early detection of aberrant crypt foci (ACF) and elimination of aberrant cells, which may progress into adenomas and adenocarcinomas in CRC. However, when a chronic inflammation persists, it creates an environment that out-competes immune surveillance mechanisms and creates a microenvironment that favors inhibition of anti-tumor immune responses and leads to formation of tolerogenic DCs and infiltration of T regulatory cells (T reg), which help in establishment of tumor cell growth. T reg infiltration is associated with bad prognosis (Erdman et al. 2005; Dunn et al. 2004). Thus, it is necessary to design drugs and standardize doses that will inhibit only tumor-promoting immune responses and will spare tumor-inhibiting responses.

## **2.5 Resolution of Inflammation and Pro-Inflammatory Mediators in CRC**

Macrophages accumulate at the site of inflammation or injury and are activated by the cytokines interleukin-10 (IL-10), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and monocytes chemoattractant protein (MCP-1). Neutrophils follow for resolution of inflammation. Eventually, fibroblasts play a role in tissue repair by secreting pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), which will help in the neutrophil response. Epithelial cells and stromal cells together help in repairing/healing the wound. Resolution of inflammation by recruitment of neutrophils can lead to complete remission of inflammation and stop the aberrant proliferation, which can extend into tumor growth. However, if this active process of resolution of inflammation is impaired, the on-going tissue repair eventually may lead to chronic inflammation, which predisposes to cancer. Cyclooxygenase (COX) and lipoxygenase (LOX) pathways and persisting inflammatory cells are involved in generating pro-inflammatory lipid mediators and gene responses, creating a favorable microenvironment that eventually can lead to tumor cell growth, proliferation, and metastases.

## **2.6 Role of Inflammatory Bioactive Arachidonic Acid Lipid Metabolites in CRC**

Arachidonic acid (AA) is an omega-6 polyunsaturated fatty acid (PUFA) present in the phospholipids of cell membranes. It acts as a precursor for production of various eicosanoids usually generated by three enzymes: COX, LOX, and cytochrome p450. The metabolites formed by action of COX and LOX are prostaglandin I<sub>2</sub>

(PGI<sub>2</sub>), prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), PGE<sub>2</sub> and thromboxane A<sub>4</sub> and Leukotrienes (LT)-A<sub>4</sub>, C<sub>4</sub>, D<sub>4</sub> and B<sub>4</sub>, respectively. The LOX metabolites involve hydroperoxy-eicosatetraenoic acids (HPETE) such as 5-HPETE, 12-HPETE, and 15-HPETE. The metabolites of P450 are epoxy-eicosatrienoic acids (EETs) resulting in four regioisomeric EETs (5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET). The other metabolites that play a role during inflammatory processes are omega-3FAs [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] derived from PUFAs. The other important bioactive molecules derived from these intermediates are lipoxins (LX) and resolvins (RVs). Unlike some of the COX and LOX metabolites, these bioactive molecules display potent anti-inflammatory, immunoregulatory, pro-resolving and anti-tumorigenic properties.

### ***2.6.1 Role of Inflammatory Lipid Molecules Derived from the COX-2 Pathway in CRC***

COX-2 is the inducible COX gene that mediates prostaglandin synthesis and pro-inflammatory functions. The expression of COX-2 is elevated in 50 % of adenomas and in 85 % of adenocarcinomas. In human intestinal tumors, COX-2 is expressed in epithelial and stromal cells; it usually is induced by interleukin 1 $\beta$  (IL-1 $\beta$ ) and TNF- $\alpha$ . Over-expression of COX-2 increased azoxymethane (AOM)-induced tumor formation (Al-Salihi et al. 2009); and COX-2 deficiency significantly diminished tumorigenesis in mouse models of colon cancer (Chulada et al. 2000a, b; Oshima et al. 1996a, b), confirming a role for COX-2 in tumorigenesis. The pro-inflammatory and pro-tumorigenic effects of COX-2 are mediated by its major end product, PGE<sub>2</sub>, which stimulates tumor cell proliferation/growth, angiogenesis, and survival and inhibits apoptosis in CRC (Wang and Dubois 2006; Castellone et al. 2006). PGE<sub>2</sub> activates a number of oncogenic signaling pathways, including  $\beta$ -catenin/transcription factors (TCF), Ras, and the phosphatidylinositol 3-kinase (PI3K) pathways. The generation of microsomal prostaglandin E synthase (mPGES-1)-deficient mice has revealed a dominant role of this enzyme in PGE<sub>2</sub> generation relevant to promotion of inflammation (Trebino et al. 2003). The mPGES-1-derived-PGE<sub>2</sub> exhibits similar inflammatory responses during tumor growth. mPGES-1 deficiency was linked to reduced vascular endothelial growth factor (VEGF). Together, these findings show that deletion or inhibition of mPGES-1 markedly reduces inflammatory responses in mouse models and eventually may lead to inhibition of tumor cell proliferation.

PGD<sub>2</sub>, another important metabolite of COX-2, appears to be a negative regulator of tumorigenesis; it has been demonstrated to possess anti-tumor properties (Murata et al. 2008). It is produced locally by inflammatory cells at sites of inflammation; and its receptor (DP1) also is expressed highly in tumor endothelial cells. The DP1 receptor is expressed on DCs that play a key role in initiating an adaptive immune response to foreign antigens (Hammad et al. 2003). These studies suggest that different COX-2-derived prostaglandins have opposing effects on

inflammation and tumor cell proliferation and that selective modulation of these prostaglandins may prevent tumor growth in CRC.

### ***2.6.2 Role of Lipid Molecules Derived from the LOX Pathway in Inflammation and CRC***

Among the LOX pathways, 5-LOX and 12-LOX pathways are closely related to inflammation and carcinogenesis; however, metabolites from another LOX pathway, 15-LOX are linked positively and shown to inhibit inflammation and carcinogenesis. A number of reports suggest the involvement of 5-LOX in early stages of CRC (Qiao et al. 1995; Bortuzzo et al. 1996; Avis et al. 2001; Ding et al. 2003; Tong et al. 2005). Hong et al. (1999) reported high expression of 5-LOX and 5-LOX-activating protein in cancer cell lines. High expression of 5-LOX and its receptors was observed in CRC patients showing poor prognosis (Ohd et al. 2003). Accumulation of 5-HETE and LT upon activation of 5-LOX resulted in cancer cell proliferation (Ding et al. 1999). COX and LOX pathways are both linked in such a way that disturbance in one pathway may lead to over-expression of the other pathway; thus, balanced inhibition of these two pathways is favorable for inhibiting CRC (Byrum et al. 1997; Griffiths et al. 1997; Goulet et al. 1994). Many studies have suggested that removal of 5-LOX and 5-LOX-activating protein (FLAP) results in increased expression of COX metabolites (Byrum et al. 1997; Goulet et al. 1994). These studies provide evidence for an important role of 5-LOX in CRC and suggest the potential for chemoprevention and treatment for CRC. Thus, targeting both COX-2 and 5-LOX pathways together and increasing production of LX and RVs is a better approach for prevention/treatment of CRC.

### ***2.6.3 Role of Reactive Oxygen and Nitrogen Species in Inflammation and CRC***

Inflammation also is associated with generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Free radicals are known to be involved in carcinogenesis (Goldstein and Witz 1990; Cerutti 1985). Inflammatory phagocytic cells make ROS.  $O_2^-$  is the initial ROS and undergoes sequential metabolic changes that generate other species (i.e., OH,  $OCI^-$ , and  $H_2O_2$ ). Usually, these reactive species lead to mutations in DNA that may be mutagenic and involved in the etiology of cancer (Babbs 1990). A significant increased expression in ROS was reported by Haklar et al. (2001) in patient colon tumors.

The literature in this area generally is consistent with the view that the enhanced production of ROS and bioavailability of nitric oxide (NO) that accompany an inflammatory response play pivotal roles in mediating formation of microvessels during tumor growth. Activated inflammatory cells produce ROS and reactive nitrogen

intermediates that can induce DNA damage and mutation in adjacent epithelial cells. These changes can stimulate ROS production within the epithelial cells may cause epigenetic silencing of tumor suppressor genes (Meira et al. 2008; Westbrook et al. 2009). The discovery of NO as a product of immune system cells has implicated this chemical in the mechanism of carcinogenesis (Tamir and Tannenbaus 1996). Produced NO can interact with  $O_2^{\cdot-}$  resulting in the propagation of the highly reactive species peroxynitrite (Oshima and Bartsch 1994). Peroxynitrite, which is formed from the reaction between  $O_2^{\cdot-}$  and NO, reacts with all classes of biomolecules and thereby is thought to be involved in many pathologic phenomena (Bartosz 1996). NO and peroxynitrite concentrations were reported to be increased in cancerous samples (Haklar et al. 2001). NO is produced by three isoforms of nitric oxide synthase (NOS) [neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), and inducible NOS (iNOS)]. nNOS and eNOS are constitutive NOS isoforms, whereas iNOS is induced upon exposure to inflammatory stimulation. iNOS is expressed in many cells: extravascular resident leukocytes (macrophages), intravascular and/or infiltrating leukocytes (neutrophils and monocytes), endothelium, and parenchymal cells, including intestinal epithelium. Its production is stimulated by lipopolysaccharide (LPS), TNF- $\alpha$ , or interleukin-1 $\beta$  (IL-1 $\beta$ ). The role of NO in colon cancer is controversial. Increased production of NO for a limited time is considered to produce positive results in inhibiting CRC, whereas chronic and continuous production of NO produced by iNOS is implicated in neoplastic transformation, a very crucial step during carcinogenesis. Studies with iNOS knockout mice suggested a positive role for iNOS in inducing polyps in adenomatous polyposis coli (APC) min/+ mice (Hofseth et al. 2003; Crowell et al. 2003; Ahn and Ohshima 2001; Nam et al. 2004). High expression of iNOS in CRC xenografts suggested an inhibitory role for iNOS in tumor growth (Xu et al. 2002). In various preclinical studies in animal models, we noted that iNOS inhibitors show promise for inhibiting CRC (Rao et al. 1999, 2002). In summary, both increased expression and decreased expression of NO is observed to have beneficial effects on CRC. Carefully designed, detailed studies to understand the role of NO during inflammation are necessary in order to understand how to modulate its effects in CRC. Interactions between NO and COX-2 are well documented, and combinations of iNOS inhibitors and COX-2 inhibitors have provided inhibition of invasive adenocarcinomas in animal models of colon cancer (Janakiram and Rao 2012).

## 2.7 Anti-inflammatory Agents in Prevention of CRC

Since it is evident that inflammation is a significant contributor to CRC, anti-inflammatory agents, both from synthetic and natural origin, have gained importance for use in prevention and treatment of CRC. As mentioned previously, NSAIDs are the main anti-inflammatory agents shown to possess anti-tumorigenic properties. They function by inhibiting AA-related pathways and by enhancing immune responses against tumor development. iNOS inhibitors also have gained importance as anti-inflammatory agents in CRC and in other cancers. CRC is associated with lower consumption of fruits and vegetables and greater consumptions of fatty foods implicated



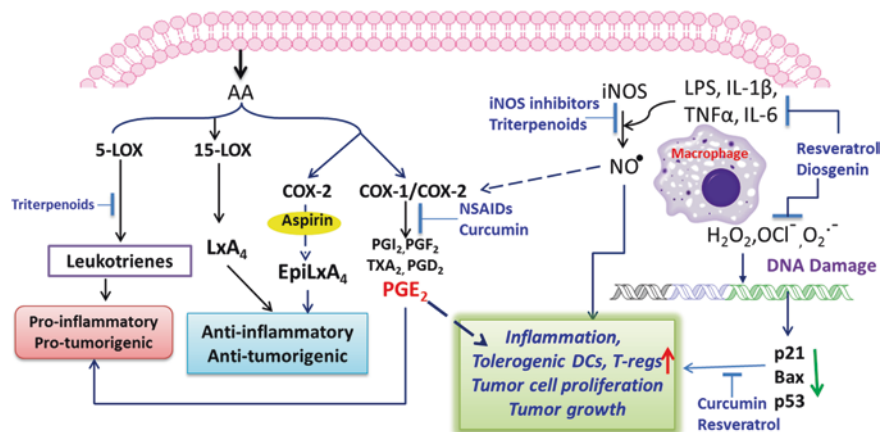
in causing CRC, thus natural constituents in these and other foods may contribute to reduce cancer risk or prevention. Here, we discuss various synthetic and natural bioactive compounds that can activate or deactivate signaling cascades implicated during tumor development and that may exhibit chemopreventive properties.

### ***2.7.1 Synthetic Anti-Inflammatory Agents for Prevention of CRC***

Preclinical and clinical evidences suggest the presence of high levels of prostaglandins, such as PGE<sub>2</sub> as mentioned earlier, which affect tumor cell proliferation by suppressing immune responses (Marnett 1992; Smith 1992). Hence, it is reasonable to use NSAIDs that can suppress the synthesis of these prostaglandins by inhibiting COX enzymes may in turn suppress tumor development and growth in colon.

Epidemiological studies, intervention trials, and animal studies have provided compelling data for inhibition of colorectal carcinogenesis by aspirin and other NSAIDs (Giovannuci 1999; Brown and Dubois 2005). The first epidemiological report suggested use of aspirin to decrease risk for CRC (Kune et al. 1988). Most of the subsequent case–control studies and prospective studies supported these results (Thun et al. 1991; Freedman et al. 1998; La Vecchia et al. 1977; Muscat et al. 1994; Peleg et al. 1994; Suh et al. 1993; Giovannucci et al. 1994; Schreinemachers and Everson 1994; Chan et al. 2005). The relative risks were very consistent in reducing the risk to 50 % in aspirin users compared with non-aspirin users. Studies on precursors of CRC such as adenomatous polyps have shown similarly decreased risks (Suh et al. 1993; Greenberg et al. 1993; Logan et al. 1993; Martinez et al. 1995). Whereas, the risk reduction of CRC is linked to the dose intake and also the duration of aspirin use which is explored in a subset of studies. Across-study comparisons indicate a dose—response relationship between aspirin and CRC or other cancer types (Harris et al. 2005). A greatest risk reduction was seen among women who took more than two aspirin tablets daily reported by the Nurses' Health Study support a strong dose—response relationship with colon cancer (Chan et al. 2005). Ten years of consistent aspirin use seems to be having reduced risks of CRC which is evidenced and seems consistent (Chan et al. 2005). Further, the role of NSAIDs/aspirin use is substantially strengthened in secondary prevention for reduction of metachronous lesions among patients with primary colorectal adenomas or CRC by two randomized controlled trials. In this trial, aspirin had a modest effect on patients with previous adenomas in reducing the risk of developing new adenomas or cancer that differed by dose. In this study, a lower dose (81 mg/day) showed better response of 19 % reduced risk of adenomas than a higher dose (325 mg/day) (Sandler et al. 2003; Baron et al. 2003). Aspirin use as a chemopreventive agent is strongly supported by these trails against colorectal carcinogenesis among individuals with a known increased risk as a result of previous disease.

We and others have previously shown that several COX inhibitors, such as indomethacin, piroxicam, aspirin, ibuprofen, and sulindac suppress colon carcinogenesis in AOM-induced F344 rats (Reddy et al. 1993, 1987; Metzger et al. 1984; Narisawa



**Fig. 2.1** The figure depicts the various pathways observed which initiates inflammation and tumor cell proliferation. Arachidonic acid metabolism leads to formation of both pro-inflammatory and anti-inflammatory metabolites. 5-LOX pathway leads to formation of leukotrienes which are known for their pro-inflammatory and pro-tumorigenic role. Triterpenoids are reported to show inhibitory effects on formation of leukotrienes. COX-2 in the presence of aspirin will lead to formation of epilipoxins (*epiLXA<sub>4</sub>*), which are anti-inflammatory and anti-tumorigenic in functions. Naturally 5-LOX pathway leads to formation of lipoxins, which show similar functions as that of *epiLXA<sub>4</sub>*. COX-1 and COX-2 pathway leads to the formation of eicosanoids, PGI<sub>2</sub>, PGF<sub>2</sub>, TXA<sub>2</sub>, PGD<sub>2</sub>, and PGE<sub>2</sub>. PGE<sub>2</sub> has been found to play a vital role during inflammation, development of Tregs, formation of tolerogenic DCs, tumor cell proliferation, and growth. NSAIDs and natural agent like curcumin are reported to have inhibitory effects on the formation of eicosanoids. LPS, IL-1β, and TNF-α are known to be involved in formation of nitric oxide (NO), and IL-6 is a known inflammatory cytokine involved during tumorigenesis. NO formed can initiate the inflammation and tumor formation by itself or by interacting COX-2 pathway. iNOS inhibitors and triterpenoids are shown to inhibit NO formation, and resveratrol and diosgenin inhibit pro-inflammatory cytokines. The free radical formation by macrophages which can cause DNA damage and eventually tumor cell proliferation by down-regulating p21, p53, and BAX are observed. Curcumin and resveratrol are reported to restore p21, p53, and BAX and inhibit tumor cell proliferation

et al. 1993; Pollard and Luckert 1984; Moorghen et al. 1988). Indomethacin was reported to inhibit tumor growth in chemically induced large-bowel tumors in rats (Kudo et al. 1980). Similar results were observed in other preclinical studies (Pollard and Luckert 1980, 1981; Narisawa et al. 1981). Due to GI toxicities of indomethacin, we developed and tested a potentially less toxic derivative, NO-indomethacin, in AOM-induced carcinoma models. Nitric oxide-releasing non-steroidal anti-inflammatory drugs (NO-NSAID) are promising chemoprevention agents; unlike conventional NSAIDs, they seem to be free of appreciable adverse effects, while they retain the beneficial activities of their parent compounds. NO-indomethacin significantly suppressed AOM-induced tumor multiplicity and incidence in F344 rats (Rao et al. 2006). Its activity is related to suppression of COX, iNOS, and β-catenin levels (Fig. 2.1).

Rigau et al. (1991) have demonstrated that colon mucosal samples from patients on long-term sulindac therapy have a reduced PG-biosynthetic capacity. In a randomized, placebo-controlled, double-blind crossover study in patients with familial adenomatous polyposis (FAP), administration of sulindac at a dose of 300 mg/day for 6–12 months caused disappearance of all polyps (Laybayle et al. 1991). Most of the clinical trials with FAP patients on long-term treatment with sulindac reported a reduction in the number and size of adenomas (Belliveau and Graham 1984; Waddel et al. 1989; Laybayle et al. 1991; Spagnesi et al. 1994; Winde et al. 1993; Giardello et al. 1993). Dietary administration of sulindac inhibited dimethyl hydrazine (DMH)-induced colon tumor incidence and multiplicity in mice (Moorghen et al. 1998; Skinner et al. 1991). In these experiments, sulindac was administered along with DMH throughout the study; however, administration of sulindac to mice seventeen weeks after DMH administration showed no reduction in colon tumor growth or development. Oral administration of sulindac (10 mg/kg) twice daily inhibited DMH-induced primary colon tumor development and growth in rats. Ahnen et al. (1994) showed that dietary administration of sulindac and its metabolite sulindac sulfone significantly inhibited AOM-induced colon carcinogenesis in F344 rats. We found that sulindac was effective at both initiation and postinitiation stages of colon tumor formation in F344 rats (Rao et al. 1995). This study suggested that its inhibitory function may be due to its modulatory effects on AA metabolism. In another study by Suh et al. (2011), the NSAIDs sulindac and naproxen, individually and in combination with atorvastatin, caused significant reduction in AOM-induced colon tumors in F344 rats. The NSAID-fed animals showed reduction in inflammatory markers iNOS and COX-2 as well as in phospho-p65 and in the pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and interleukin 4 (IL-4). Hence, use of NSAIDs in combination with statins was suggested for retaining efficacy with less/no GI toxicity.

The concern over gastric toxicity associated with aspirin use led to efforts to develop COX-2-specific inhibitors (COXibs such as rofecoxib, celecoxib) (Gupta and Dubois 2001). Available literature provides strong evidence for a role of COX-2 in inflammation and carcinogenesis. Several studies using COX-2 knock-out or disrupted genes in mouse models of FAP or in chemically induced colon carcinogenesis in rats indicated that COX-2 selective inhibitors, such as rofecoxib and celecoxib, inhibit formation of intestinal adenomas (Dannenberget al. 2005; Rao and Reddy 2004; Oshima et al. 1996a, b, 2001; Chulada et al. 2000a, b; Jacoby et al. 2000; Boolbol et al. 1996; Mahmoud et al. 1998; Kawamori et al. 1998; Reddy and Rao 2002). In a clinical trial, FAP patients treated twice daily with 400 or 200 mg celecoxib had 31 and 12 % reduction, respectively, in polyp number (Arber et al. 2006). In this clinical trial, celecoxib, at a dose of 400 mg daily, reduced advanced adenoma formation in the colon by almost 50 % compared with the placebo through a 3-year treatment period (Arbor et al. 2006). Although introduction of COXibs was successful in reducing GI toxicity, these drugs were associated with cardiovascular toxicity due to high selectivity toward COX-2 (Smith et al. 2000; Silverstein et al. 2000; Laine et al. 2003; Bresalier et al. 2005; Nussmeier et al. 2005; Solomon et al. 2005). An initial study indicated

possible increases in the incidence of myocardial infarction with use of COXibs (Bombardier et al. 2000). No randomized controlled trials specifically to address the issue of cardiovascular toxicity were conducted; but trials were initiated to test the efficacy of COXibs in the prevention of metachronous colonic polyps (Bresalier et al. 2005; Solomon et al. 2005) and the management of postoperative pain (Nussmeier et al. 2005). The above-mentioned trials suggested that patients using these COX-2 inhibitors were showing cardiovascular events. These observations led to temporary withdrawal of COXibs from the US market in 2004. The findings suggest that this cardiovascular toxicity is specific to this class of drugs; but aspirin and other non-specific COX-2 inhibitors still have potential for chemoprevention of CRC.

In February 2005, the Food and Drug Administration (FDA) Advisory Committee meeting recommended that COXibs remain on the market, but with warnings added to labels (Alberts et al. 2005). The committee agreed that since celecoxib is the least likely to be associated with adverse cardiovascular events, it is the most appropriate COXib to study for the prevention and treatment of cancer. Complicating the risk—benefit evaluation are individual differences in both cancer risk and sensitivity to toxic events. The development of very low non-toxic doses of COXibs or COX and LOX-inhibiting regimens in combination with other agents continues. Licofelone (ML3000) is the first member of a new dual COX/5-LOX inhibitor class and currently is under evaluation as a treatment for osteoarthritis. A multicenter study explored the effects of licofelone in comparison with naproxen as a disease-modifying agent and showed beneficial effects on cartilage. Although phase III trials have been completed successfully, no dates for regulatory submission have been given for this drug. Its safety profile shows fewer GI events than NSAIDs and selective COX-2 inhibitors (Martel-Pelletier et al. 2003; Cicero et al. 2005; Moreau et al. 2005; Bias et al. 2004). We tested licofelone in APC<sup>Min/+</sup> mice and found it to possess potential chemopreventive properties (Mohammed et al. 2011). The efficacy achieved with licofelone was comparable to or more effective than several NSAIDs and the COX-2-selective inhibitors celecoxib and rofecoxib (Swamy et al. 2006; Jacoby et al. 1996; Rao and Reddy 2004; Rao et al. 2000; Orner et al. 2003). This result suggests that a balanced inhibition of COX and LOX pathways is a better approach to obtain diminished side effects with high efficacy. The beneficial effects of NSAIDs in chemoprevention of CRC suggest that inflammatory mechanisms are playing a vital role in tumor development, with strongest for colorectal cancer. Future work to understand the molecular mechanisms still is needed to establish the chemopreventive potential of NSAIDs for use as a preventive for and treatment of CRC.

### ***2.7.2 Role of iNOS Inhibitors in Prevention of CRC***

High NOS activity and high levels of NO are observed in AOM-induced colonic tumors in rats (Rao et al. 1999, 2002), in Crohn's disease (Rachmilewitz et al. 1995)

and in ulcerative colitis (Colon et al. 2000). Over-expression of NO was observed in preneoplastic colon lesions and also in human colon adenocarcinomas (Hao et al. 2001; Yagihashi et al. 2000; Lagares-Garcia et al. 2001; Szaleczky et al. 2000). High iNOS levels were observed in colons of animals fed a high-fat diet, suggesting a role of high-fat diets in inducing inflammatory conditions and CRC in humans (Wan et al. 2000). Collectively, these studies support a positive role of iNOS in inducing CRC and use of iNOS-inhibiting agents for suppressing the iNOS activity and its tumorigenic effects (Fig. 2.1).

*S,S*-1,4-phenylene-*bis*(1,2-ethanediy)l*bis*-isothioureia, PBIT a selective iNOS inhibitor, caused suppression of ACF development in rats by reducing protein levels of j; and iNOS in colonic mucosa (Rao et al. 1999). Kawamori et al. (2000) reported similar results with *L*-nitro arginine methyl ester (L-NAME), an *L*-arginine inhibitor on the development of ACF induced by AOM in rats. Animals that received 100 ppm of L-NAME for 11 weeks showed 32 % inhibition of ACF multiplicity. To increase the potency of PBIT with lower concentrations, a isosteric analog of PBIT, selenium [*S,S'*-1,4-phenylene*bis*(1,2-ethanediy)l *bis*-isothioureia] (Se-PBIT) was developed and tested recently, in colon cancer animal model. We reported chemopreventive properties of Se-PBIT on ACFs induced by AOM in rats (Janakiram et al. 2013). We have studied extensively the role of iNOS inhibitors in colon carcinogenesis (Rao et al. 2002; Rao 2004). We tested iNOS-selective inhibitors individually and in combination with COX inhibitors and found that low-dose combinations of the COX-2 inhibitor celecoxib and the iNOS inhibitor SC-51 inhibited AOM-induced crypt formation in rats (Rao 2004). L-NAME and iNOS-specific inhibitors have been reported to have inhibitory effects on formation of adenomas, adenocarcinomas (Kawamori et al. 2000; Schleiffer et al. 2000), and adenomatous polyps (Ahn and Ohshima 2001). NO signaling cascades also are involved in the migration of tumor cells. More detailed studies into role of NO at different doses and during different stages of tumor development are necessary for design of better iNOS inhibitors for prevention and treatment of CRC.

### 2.7.3 *Natural Anti-Inflammatory Agents for Prevention of CRC*

Epidemiologic evidence supports the benefit of changes in dietary and exercise patterns for CRC prevention. Among well-known dietary agents, curcumin has been valued for more than 5,000 years for its medicinal properties and for its warm, peppery flavor. Curcumin is one of the curcuminoids of turmeric. Curcumin is a highly pleiotropic molecule capable of interacting with numerous molecular targets involved in inflammation. It is reported to interact with inflammatory processes by down-regulating COX-2, 5-LOX, iNOS and also production of inflammatory cytokines such as TNF- $\alpha$ , IL-1, -2, -6, -8 and -12, and also macrophage inhibitory protein (MIP), monocytes chemoattractant protein (MCP), and matrix metalloproteinases (MMPs) (Goel et al. 2008; Abe et al.

1999) (Fig. 2.1). Curcumin was found to be effective in reducing colitis induced by 1,4,6-trinitrobenzene sulfonic acid. Ukil et al. (2003) reported reduced levels of NO and O<sub>2</sub> radicals and suppression of NF- $\kappa$ B activation in curcumin-treated colonic mucosa. We reported chemopreventive properties of curcumin (2,000 ppm) in inhibiting development of ACF and colon adenocarcinomas in AOM-induced F344 rats (Rao et al. 1995). Dietary administration of curcumin resulted in >50 % inhibition of AOM-induced colon adenocarcinoma incidence and multiplicity. Kawamori et al. (1999) reported a 78 % suppression of progression from adenoma to adenocarcinoma in a preclinical animal model with a high dose (6,000 ppm) of curcumin. Curcumin has been tested in combination with green tea catechins, another class of natural agents, and also in combination with the synthetic COX-2 inhibitor celecoxib for increased efficacy with low doses or to increase its bioavailability for better efficacy (Xu et al. 2010; Shpitz et al. 2006).

Although curcumin is well known for its anti-inflammatory and anti-tumorigenic properties in preclinical animal models, the absorption required for achieving its anti-tumor properties is still a concern. Clinical trials to assess pharmacokinetics, metabolism, and systemic bioavailability in cancer patients are inconclusive. Cheng et al. (2001) conducted a phase I clinical trial on cancer patients and reported poor absorption and minimal serum concentrations of curcumin. Another phase I study reported similar poor absorption of curcumin in patients (Sharma et al. 2004). However, Garcea et al. (2005) reported pharmacologically efficacious levels of curcumin ( $12.7 \pm 5.7$  nmol/g) in both malignant colorectal tissue and normal colorectal tissue ( $7.7 \pm 1.8$  nmol/g) from CRC patients, suggesting a potential anti-tumorigenic role for curcumin in CRC. Three other clinical trials have investigated the use of curcumin (curcumin, demethoxycurcumin, or bisdemethoxycurcumin) therapy in patients with established CRC and reported a decrease in carcinogenic embryonic antigen and PGE<sub>2</sub> levels (Sharma et al. 2001, 2004). Another trial of curcumin in CRC patients required high doses (3.6 g daily) to observe any effects on oxidative DNA adducts, and COX-2 markers (Garcea et al. 2005). In that study, no change in COX-2 protein was observed. Additional studies are in progress to develop curcumin formulations, analogs, and tumor site delivery methods to increase bioavailability for prevention and treatment of CRC.

Piperine, the principle bioactive compound of *Piper nigrum* and *Piper longum*, is included in many traditional formulae to enhance the effectiveness of other bioactive compounds, such as curcumin. Piperine has been reported to have immunomodulatory, anti-carcinogenic, anti-asthmatic, stimulatory, hepatoprotective, and anti-inflammatory (Darshan and Doreswamy 2004). It was found to be genotoxic but had no adverse effects when tested for toxicity profile in rats at doses 5–20 times the normal human intake (Bhat and Chandrasekhara 1986; Piyachaturawat et al. 1983). Due to its apolar nature, piperine alters lipid dynamics and it changes the conformation of enzymes in the intestine. Due to its unique properties, it is used in combinations to enhance the bioavailability of the other drugs. Its potential to increase the bioavailability of drugs in humans is of great clinical significance (Bajad et al. 2003). Nalini et al. (2006) reported inhibitory effects of piperine

on DMH-induced colon tumors in F344 rats. We reported inhibitory effects of piperine on AOM-induced colon tumors in F344 rats. The potential of piperine to enhance the bioavailability of other potent drugs is an important property that can be exploited to increase the efficacy of agents that inhibit CRC.

Another bioactive molecule available in edible fruits is resveratrol. The chemopreventive function of resveratrol was reported by Jang et al. (1997). They showed that it inhibited cellular events associated with the initiation, promotion, and progression of cancer development. A clinical trial with whole grape extract in patients with colon cancer resulted in reduced the expression of Wnt target genes in normal mucosa with no change in colon tissue (Nguyen et al. 2009). These trial results need more careful evaluation because of lack of control for dietary intake of resveratrol-rich food and the absence of control for ingestion of confounding medications. A second trial in 20 selected histologically confirmed CRC patients administered trans-resveratrol during 8 days prior to surgical resection reported a 5 % ( $p = 0.05$ ) reduction in cell proliferation (Patel et al. 2010). The cell proliferation analysis was carried out preintervention and postintervention with resveratrol in tissue samples. These data suggest achievement of high enough concentrations of resveratrol in the intestinal tissues to show some inhibition of cell proliferation. However, some preclinical and clinical studies suggest that bioavailability of resveratrol is low due to poor absorptions as a result of intestinal metabolism and low activity of ATP-binding cassette (ABC) transporters (Juan et al. 2010, 2012; Alfaras et al. 2010a; Walle 2011; Cottart et al. 2010). In a preclinical animal model in which ACF are induced by DMH, an oral dose of 60 mg/kg resveratrol caused 50 % inhibition in the medial and 48 % inhibition in the distal tumors in rats (Alfaras et al. 2010b). Resveratrol also was observed to have inhibitory effects on mucin-depleted foci (MDFs) with reduction of the number of MDFs by 36 and 53 % in the medial and distal colon, respectively (Alfaras et al. 2010b). It also was found to be effective in long-term preclinical assays with development of adenocarcinomas as an end point. Oral administration of resveratrol (0.2 mg/kg in drinking water) for 100 days showed reduced ACFs and colon carcinogenesis in F344 rats (Tessitore et al. 2000). This reduction probably was due to modulation of Bax and p21, which regulate cell proliferation and apoptosis (Fig. 2.1). Daily administration of 8 mg/kg of trans-resveratrol for 30 weeks in DMH-treated rats resulted in reduction in the incidence and multiplicity of ACFs and also decreased formation of multicrypt (more than 6) ACFs (Sengottuvelan and Nalini 2006). Inhibition of ACFs with 6 or more crypts is an indication of potent chemopreventive efficacy via suppressing the progression of preneoplastic lesions to neoplasia. These results suggest that resveratrol possesses chemopreventive properties and can suppress the progression of preneoplasia to malignant neoplasia in colon. This study also reported inhibitory effects of resveratrol on polyamine synthesis, which is high in neoplastic tissues (Sengottuvelan and Nalini 2006).

Resveratrol also has been evaluated for its anti-tumor activity in genetically modified mice. Resveratrol (0.01 % in drinking water) decreased the number of tumors in the small intestine and completely suppressed tumor formation in the colon of APC<sup>Min/+</sup> mice (Schneider et al. 2001). In contrast to these results,

Ziegler et al. (2004) reported null results with resveratrol in APC<sup>Min/+</sup> mice. Even though this study used high doses of resveratrol (4, 20, and 90 mg/kg body weight) in pellet form, no difference was observed in incidence of intestinal tumors. Another Apc min mouse study reported a 27 % decrease in adenoma formation by 60 mg/kg of resveratrol administered in the diet (Sale et al. 2005). This reduction was attributed to decreases (of 58 and 62 % compared with intestinal mucosa from mice on control diet) in PGE<sub>2</sub>, which is involved in the maintenance of the malignant phenotype (Sale et al. 2005). Evidence from all of these studies suggests that resveratrol has potential prevention and therapeutic properties and needs further evaluation for its dosage and clinical efficacy in CRC.

Diosgenin, a natural steroidal saponin found predominantly in fenugreek and wild yams, has diverse biological properties (Raju and Mehta 2009). The commercial synthesis of steroid products, such as cortisone, pregnenolone, and progesterone, involves use of diosgenin as a precursor (Raju and Mehta 2009). It is considered safe since it is neither synthesized nor metabolically converted into steroid by-products in the mammalian body. In preliminary studies with human subjects, diosgenin has been found to be effective against hyperglycemia (McAnuff et al. 2005), hypercholesterolemia (Juarez-Oropeza et al. 1987; Son et al. 2007), and hypertriacylglycerolemia (Kwon et al. 2003). Significant anti-inflammatory functions have been demonstrated in relevant animal models. It is used in rats to heal the GI toxicity generated by indomethacin treatment. Its anti-inflammatory role has been explored further by Yamada et al. (1997). Preclinical animal studies with AOM-induced ACFs in F344 rats suggested that diosgenin possesses chemopreventive efficacy in CRC. Administration of 0.1 or 0.05 % diosgenin in the diet during initiation, postinitiation, or promotion stages of colon carcinogenesis dose-dependently decreased ACF formation (Raju et al. 2004). Another study investigated the preventive effects of diosgenin (20, 100, or 500 mg/kg) on AOM/dextran sodium sulfate (DSS)-induced CRC in mice. Diosgenin at very low doses significantly inhibited (53, 46, and 40 %, respectively) colonic mucosal ulcers and dysplastic crypts induced by AOM/DSS treatment and also reduced expression of inflammatory cytokine genes, including IL-1 $\beta$ , IL-6, IL-12b, and TNF- $\alpha$ , which are significantly elevated in the colonic mucosa of mice treated with AOM/DSS (Fig. 2.1). These studies suggest that diosgenin is a potent bioactive molecule possessing both anti-inflammatory and anti-tumorigenic properties that make it ideal for further investigation of its molecular and anti-neoplastic functions in human clinical trials.

Triterpenoids are isolated from various medicinal plants and have been studied for their anti-inflammatory properties. Mostly these compounds are non-toxic and have made their way into cosmetics and health products (Liu 1995). Recently, interest in understanding and elucidating the biological roles of triterpenoids for their hepatoprotective, analgesic, anti-tumor, anti-inflammatory, and immunomodulatory effects is increasing (Mahato and Sen 1997; Liu 1995). These agents are broken down in the gut to release triterpene metabolites, which are integrated into the intestinal cell membranes, absorbed, and lead to modulation of signaling pathways. These molecules inhibit expression of inflammatory genes such as



COX-2, iNOS and various inflammatory cytokines that are known enhancers of carcinogenesis (Janakiram et al. 2008; Rao et al. 2002, Raju et al. 2004) (Fig. 2.1). Recently, triterpene analogs that are more potent than the original parent molecules have been synthesized. Kawamori et al. (1995) found that oleanolic acid (ONA), a crude plant extract of triterpenoid at a dose of 200 ppm, was effective in reducing ACF in the intestine of F344 rats. We reported the anti-neoplastic properties of ONA and the analog 18 $\alpha$ -olean-12-ene-3  $\beta$ -23,28-triol (OT) in AOM-induced ACFs in F344 rats (Janakiram et al. 2008). These triterpenoids significantly suppressed carcinogen-induced colonic preneoplastic lesions at dietary doses of 750 and 1,500 ppm of ONA, and 250 and 500 ppm of OT and without any toxicity. ONA inhibited 52 % of total AOM-induced ACFs and ~66 % of ACF with four or more crypts. OT inhibited up to 48 % of total AOM-induced ACF formation and 60 % of ACF with four or more crypts at very low doses compared with those of ONA. These studies support chemopreventive effects of triterpenoids in CRC and suggest that an in-depth evaluation of these agents in clinical trials should be carried out to assess pharmacokinetics, bioavailability, and anti-neoplastic functions.

Epidemiological, experimental, and clinical studies provide evidence for anti-CRC activity of omega ( $\omega$ )-3 PUFAs. Evidence from animals and humans suggest that  $\omega$ -3 PUFAs may play an inhibitory role during different stages of CRC, from primary CRC prevention to “tertiary” prevention after treatment of CRC and advanced metastatic disease. Out of 8 reported clinical studies of  $\omega$ -3 PUFAs supplementation, 6 reported protective effects. In patients with a previous history of sporadic colorectal adenomas, oral supplementation with  $\omega$ -3 PUFA has resulted in a 13–70 % reduction in intestinal epithelial cell proliferation as compared to placebo groups (Cockbain et al. 2012). A phase III randomized, double-blind, placebo-controlled trial investigated treatment with eicosapentaenoic acid-free fatty acid (EPA-FFA) in 58 patients with FAP who had previously undergone colectomy and ileorectal anastomosis and showed a 22.4 % reduction in polyp number compared with placebo (West et al. 2010). Colon cancer xenograft studies showed consistent protective effects (40–60 % reduction in xenograft size) in mice supplemented with  $\omega$ -3 PUFAs as compared to untreated mice (Boudreau et al. 2001; Kato et al. 2002; Calviello et al. 2004). Similar beneficial results were reported from studies with CRC cell allograft tumors (Mund et al. 2007; Cannizzo and Broitman 1989; Togni et al. 2003; Pizato et al. 2005). In spite of these encouraging data, no published studies yet have investigated the anti-neoplastic effect of  $\omega$ -3 PUFAs in patients with primary or metastatic CRC.

Fish and fish oil are rich sources of the  $\omega$ -3 PUFAs EPA and DHA. The metabolites derived from these PUFAs result in formation of 3-series prostaglandins, which are anti-inflammatory rather than pro-inflammatory and also may possess anti-tumor properties. A report of a switch from 2 series PGE<sub>2</sub> to 3 series PGE<sub>3</sub> was demonstrated in colonic mucosa of rats treated with fish oil (Vanamala et al. 2008). The recently discovered anti-inflammatory lipid mediators RVs and LX derived from EPA and DHA are gaining importance for their anti-neoplastic functions. RVs derived from EPA are called as “E” series. Protectins are also generated

from precursors of omega 3-PUFAs. RVs or protectins from DHA, named “D” series, possess anti-inflammatory and immunomodulatory properties. The concentration required for these lipid mediators to exhibit any biological activity is in the nanomolar or picomolar range. Acetylation of aspirin by COX-2 in the presence of EPA results in formation of 5,12,18*R*-trihydroxy-EPA (18*R*-*RvE1*) (Janakiram et al. 2011) (Fig. 2.1). Ingestion of aspirin and EPA generated 18*R*-*RvE1* that was detectable in plasma of healthy volunteers (Oh et al. 2011). The anti-inflammatory role of *RvE1* is well documented in a mouse model of DSS-induced colitis; it acts through inhibition of phosphorylation of NF- $\kappa$ B (Ishida et al. 2010). Another study reported a protective role of *RvE1* in mouse colitis through induction of intestinal alkaline phosphatase (Campbell et al. 2010). EPA and DHA exhibited protective effects against colitis in a rat model by restoring the number of mature, mucin-filled goblet cells (Arita et al. 2005). Two other studies also reported protective effects of *RvE1* against colitis induced by DSS and 2, 4, 6-trinitrobenzene sulfonic acid (Nieto et al. 2002; Ishida et al. 2009).

Lipoxin A<sub>4</sub> (LXA<sub>4</sub>) was shown to inhibit neutrophil chemotaxis, adherence, transmigration, and activation during resolution of inflammation and suppression of IL-8 production by epithelia and leukocytes and to cause clearing of neutrophils by up-regulation of monocyte ingestion (Serhan 1997, 2002; Canny et al. 2002). Decreased LXA<sub>4</sub> expression was shown in a DSS-induced colitis model (Gewirtz et al. 2002). Protective effects of LXA<sub>4</sub> analogs were observed in DSS and other chemically induced colitis animal models (Gewirtz et al. 2002; Fiorucci et al. 2001). The protective effects of these analogs are attributed for their LXA<sub>4</sub> receptor-mediated inhibitory effects on pro-inflammatory signaling pathways. 15-*epi*-LXA<sub>4</sub> is formed in the presence of aspirin; and some of the preventive or therapeutic effects of aspirin-like NSAIDs may be through these 15-*epi*-LX (Claria and Serhan 1995) (Fig. 2.1). The anti-inflammatory functions of these lipid mediators suggest a potential chemopreventive therapeutic strategy for inflammation-related diseases like CRC.

## 2.8 Conclusions

Epidemiological and clinical literature strongly implicates chronic inflammation in neoplastic diseases, especially in CRC. Different inflammatory molecules and signals play different roles during different stages of CRC development. AA metabolism, via COX-2 and 5-LOX pathways, generates a variety of lipid mediators that affect initiation, growth, and development of CRC. Current evidence from preclinical, clinical, and epidemiological studies supports a positive role for anti-inflammatory agents, particularly NSAIDs as inhibitors of CRC; however, these drugs can have GI and cardiovascular toxicities. Additional studies are needed to design analogs or derivatives of these agents, to manipulate doses and to select appropriate patient populations to provide increased safety without losing efficacy for CRC suppression. It also is important to develop other agents that can balance COX

and LOX inhibition, including natural agents like curcumin or synthetic agents like licoferone, to achieve safer toxicity profiles while retaining significant inhibition of CRC. Additional natural bioactive anti-inflammatory compounds are being identified to provide beneficial effects against colitis-induced inflammation and CRC. Many of these agents are well tolerated and may provide safe alternatives to existing, more toxic compounds. Increased consumption of EPA- and DHA-rich foods may reduce inflammation and its related CRC conditions. And other novel lipid mediators, such as LX, RVs and their analogs, need to be evaluated in CRC models for their effects on colon mucosal immunity against development of CRC.

**Acknowledgements** We want to acknowledge the Grant support NCI-R01-94962; NCI-CN-53300 for the work quoted in this chapter and Dr. Julie Sando for her scientific and language editing of this chapter.

## References

- Abe Y, Hashimoto S, Horie T (1999) Curcumin inhibition of inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. *Pharmacol Res* 39(1):41–47
- Ahn B, Ohshima H (2001) Suppression of intestinal polyposis in Apc (Min/+) mice by inhibiting nitric oxide production. *Cancer Res* 61:8357–8360
- Ahnen D, Hixson L, Alberts D, Bogert C, Einspahr K, Brendel P, Gross N, Paranele R, Pamukeu R (1994) Sulindac and its sulfone metabolite (FGN-1) both inhibit rat colon carcinogenesis but neither inhibit colonic proliferation. *Proc Am Assoc Cancer Res* 35:631
- Alberts DS et al (2005) What happened to the COXibs on the way to the cardiologist? *Cancer Epidemiol. Biomarkers Prev* 14:555–556
- Alfaras I, Juan ME, Planas JM (2010a) Trans-resveratrol reduces precancerous colonic lesions in dimethylhydrazine-treated rats. *J Agric Food Chem* 58(13):8104–8110
- Alfaras I, Pérez M, Juan ME, Merino G, Prieto JG, Planas JM, Alvarez AI (2010b) Involvement of breast cancer resistance protein (BCRP1/ABCG2) in the bioavailability and tissue distribution of trans-resveratrol in knockout mice. *J Agric Food Chem* 58(7):4523–4528
- Al-Salihi MA, Pearman AT, Doan T, Reichert EC, Rosenberg DW, Prescott SM, Stafforini DM, Topham MK (2009) Transgenic expression of cyclooxygenase-2 in mouse intestine epithelium is insufficient to initiate tumorigenesis but promotes tumor progression. *Cancer Lett* 273(2):225–232
- Arber N, Eagle CJ, Spicak J, Rácz I, Dite P, Hajer J, Zavoral M, Lechuga MJ, Gerletti P, Tang J, Rosenstein RB, Macdonald K, Bhadra P, Fowler R, Wittes J, Zauber AG, Solomon SD, Levin B, PreSAP Trial Investigators (2006) Celecoxib for the prevention of colorectal adenomatous polyps. *N Engl J Med* 355(9):885–895
- Arita M, Yoshida M, Hong S, Tjonahen E, Glickman JN, Petasis NA, Blumberg RS, Serhan CN (2005) *Proc Natl Acad Sci* 102:7671–7676
- Askling J, Dickman PW, Karlen P et al (2001) Family history as a risk factor for colorectal cancer in inflammatory bowel disease. *Gastroenterology* 120:1356–1362
- Atreya I, Atreya R, Neurath MF (2008) NF-kappaB in inflammatory bowel disease. *J Intern Med* 263:591–596
- Atreya I, Neurath MF (2008) Immune cells in colorectal cancer: prognostic relevance and therapeutic strategies. *Expert Rev Anticancer Ther* 8:561–572
- Avis I, Hong SH, Martinez A, Moody T, Choi YH, Trepel J et al (2001) Five-lipoxygenase inhibitors can mediate apoptosis in human breast cancer cell lines through complex eicosanoid interactions. *J Fed Am Soc Exp Biol* 15:2007–2009

- Babbs CF (1990) Free radicals and etiology of colon cancer. *Free Rad Biol Med* 8:191–200
- Bajad S, Coumar M, Khajuria R, Suri OP, Bedi KL (2003) Characterization of a new rat urinary metabolite of piperine by LC/NMR/MS studies. *Eur J Pharm Sci* 19(5):413–421
- Bansal P, Sonnenberg A (1996) Risk factors of colorectal cancer in inflammatory bowel disease. *Am J Gastroenterol* 91(1):44–48
- Baron JA et al (2003) A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med* 348:891–899
- Bartosz G (1996) Peroxynitrite mediator of the toxic action of nitric oxide. *Acta Biochim Polon* 43:645–660
- Belliveau P, Graham AM (1984) Mesenteric desmoid tumor in Gardener's syndrome treated by sulindac. *Dis Colon Rectum* 27:53–54
- Bhat BG, Chandrasekhara N (1986) Studies on the metabolism of piperine: absorption, tissue distribution and excretion of urinary conjugates in rats. *Toxicology* 40(1):83–92
- Bias P, Buchner A, Klessner B, Laufer S (2004) The gastrointestinal tolerability of the LOX/COX inhibitor, licofelone, is similar to placebo and superior to naproxen therapy in healthy volunteers: results from a randomized, controlled trial. *Am J Gastroenterol* 99:611–618
- Bombardier C et al VIGOR Study Group (2000) Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. *N Engl J Med* 343:1520–1528
- Boolbol SK, Dannenberg AJ, Chadburn A et al (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 SJ, Rigas B et al (1996) The effect of leukotrienes B and selected HETEs on the proliferation of colon cancer cells. *Biochimica et Biophysica Acta* 1300:240–246
- Boudreau MD, Sohn KH, Rhee SH, Lee SW, Hunt JD, Hwang DH (2001) Suppression of tumor cell growth both in nude mice and in culture by n-3 polyunsaturated fatty acids: mediation through cyclooxygenase-independent pathways. *Cancer Res* 61(4):1386–1391
- Bresalier RS et al (2005) Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med* 352:1092–1102
- Brown JR, DuBois RN (2005) COX-2: a molecular target for colorectal cancer prevention. *J Clin Oncol* 23:2840–2855
- Byrum RS, Goulet JL, Griffiths RJ, Koller BH (1997) Role of the 5-lipoxygenase-activating protein (FLAP) in murine acute inflammatory responses. *J Exp Med* 185:1065–1075
- Calviello G, Di Nicuolo F, Gagnoli S, Piccioni E, Serini S, Maggiano N, Tringali G, Navarra P, Ranalletti FO, Palozza P (2004) n-3 PUFAs reduce VEGF expression in human colon cancer cells modulating the COX-2/PGE<sub>2</sub> induced ERK-1 and -2 and HIF-1 $\alpha$  induction pathway. *Carcinogenesis* 25(12):2303–2310
- Campbell EL, MacManus CF, Kominsky DJ, Keely S, Glover LE, Bowers BE, Scully M, Bruyninckx WJ, Colgan SP (2010) Resolvin E1-induced intestinal alkaline phosphatase promotes resolution of inflammation through LPS detoxification. *Proc Natl Acad Sci U S A* 107(32):14298–14303
- Cannizzo F Jr, Broitman SA (1989) Post promotional effects of dietary marine or safflower oils on large bowel or pulmonary implants of CT-26 in mice. *Cancer Res* 49(15):4289–4294
- Canny G, Levy O, Furuta GT, Narravula-Alipati S, Sisson RB, Serhan CN et al (2002) Lipid mediator-induced expression of bactericidal/permeability-increasing protein (BPI) in human mucosal epithelia. *Proc Natl Acad Sci* 99:3902–3907
- Castellone MD, Teramoto H, Gutkind JS (2006) Cyclooxygenase-2 and colorectal cancer chemoprevention: the beta-catenin connection. *Cancer Res* 66:11085–11088
- Cerutti P (1985) Prooxidant states and tumor promotion. *Science* 227:375–381
- Chan AT, Giovannucci EL, Meyerhardt JA et al (2005) Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. *JAMA* 294(8):914–923
- Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, Ko JY, Lin JT, Lin BR, Ming-Shiang W, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, Lai MK, Pu YS, Pan MH, Wang YJ,

- Tsai CC, Hsieh CY (2001) Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res* 21(4B):2895–2900
- Chulada PC, Thompson MB, Mahler JF, Doyle CM, Gaul BW, Lee C, Tiano HF, Morham SG, Smithies O, Langenbach R (2000a) Genetic disruption of PtgS-1, as well as PtgS-2, reduces intestinal tumorigenesis in Min mice. *Cancer Res* 60(17):4705–4708
- Chulada PC, Thompson MB, Mahler JF et al (2000b) Genetic disruption of PtgS-1 as well as PtgS-2 reduces intestinal tumorigenesis in Min mice. *Cancer Res* 60:4706–4708
- Cicero AF, Derosa G, Gaddi A (2005) Combined lipooxygenase/cyclo-oxygenase inhibition in the elderly: the example of licoferone. *Drugs Aging* 22:393–403
- Claria J, Serhan CN (1995) Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell—leukocyte interactions. *Proc Natl Acad Sci* 92:9475–9479
- Clevers H (2004) At the crossroads of inflammation and cancer. *Cell* 118:671–674
- Cockbain AJ, Toogood GJ, Hull MA (2012) Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. *Gut* 61:135–149
- Colon AL, Menchen L, Lizasoain I et al (2000) Inducible nitric oxide synthase activity is expressed not only in inflamed but also in normal colonic mucosa in patients with ulcerative colitis: a potential prognostic marker. *Am J Gastroenterol* 95:1371–1373
- Cottart CH, Nivet-Antoine V, Laguillier-Morizot C, Beaudeau JL (2010) Resveratrol bioavailability and toxicity in humans. *Mol Nutr Food Res* 54(1):7–16
- Crowell JA, Steele VE, Sigman CC, Fay JR (2003) Is inducible nitric oxide synthase a target for chemoprevention? *Mol Cancer Ther* 2:815–823
- Dannenber AJ, Lippman SM, Mann JR, Subbaramaiah K, Dubois RN (2005) Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention. *J Clin Oncol* 23:254–266
- Darshan S, Doreswamy R (2004) Patented antiinflammatory plant drug development from traditional medicine. *Phytother Res* 18(5):343–357
- Ding XZ, Iversen P, Cluck MW et al (1999) Lipooxygenase inhibitors abolish proliferation of human pancreatic cancer cells. *Biochem Biophys Res Commun* 261:218–223
- Ding XZ, Tong WG, Adrian TE (2003) Multiple signal pathways are involved in the mitogenic effect of 5(S)- HETE in human pancreatic cancer. *Oncology* 65:285–294
- Dunn GP, Old LJ, Schreiber RD (2004) The immunobiology of cancer immunosurveillance and immunoeediting. *Immunity* 21:137–148
- Erdman SE, Sohn JJ, Rao VP et al (2005) CD4CD25 regulatory lymphocytes induce regression of intestinal tumors in ApcMin/ mice. *Cancer Res* 65:3998–4004
- Feagins LA, Souza RF, Spechler SJ (2009) Carcinogenesis in IBD: potential targets for the prevention of colorectal cancer. *Nat Rev Gastroenterol Hepatol* 6:297–305
- Fiorucci S, Meli R, Bucci M, Cirino G (2001) Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy? *Biochem Pharmacol* 62:1433–1438
- Freedman AN et al (1998) Aspirin use and p53 expression in colorectal cancer. *Cancer Detect Prev* 22:213–218
- Garcea G, Berry DP, Jones DJ, Singh R, Dennison AR, Farmer PB, Sharma RA, Steward WP, Gescher AJ (2005) Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. *Cancer Epidemiol Biomarkers Prev* 14(1):120–125
- Gewirtz AT, Collier-Hyams LS, Young AN, Kucharzik T, Guilford WJ, Parkinson JF et al (2002) Lipoxin A4 analogs attenuate induction of intestinal epithelial proinflammatory gene expression and reduce the severity of dextran sodium sulfate-induced colitis. *J Immunol* 168(10):5260–5267
- Giardello FM, Hamilton SR, Krush AJ, Piantodosi S, Hylind LM, Cleano P, Booker SV, Robinson RC, Offerhaus GJA (1993) Treatment of colonic rectal adenomas with sulindac in familial adenomatous polyposis. *N Eng J Med* 328:1313–1316
- Giovannucci E (1999) The prevention of colorectal cancer by aspirin use. *Biomed Pharmacother* 53:303–308

- Giovannucci E et al (1994) Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. *Ann Intern Med* 121:241–246
- Goel A, Kunnumakkara AB, Aggarwal BB (2008) Curcumin as “Curecumin”: from kitchen to clinic. *Biochem Pharmacol* 75(4):787–809
- Goldstein BD, Witz G (1990) Free radicals and carcinogenesis. *Free Radic Res Commun* 11:3–10
- Goulet JL, Snouwaert JN, Latour AM, Coffman TM, Koller BH (1994) Altered inflammatory responses in leukotriene-deficient mice. *Proc Natl Acad Sci USA* 91(26):12852–12856
- Greenberg ER, Baron JA, Freeman DH, Mandel JS, Haile R (1993) Reduced risk of large-bowel adenomas among aspirin users. The Polyp Prevention Study Group. *J Natl Cancer Inst* 85:912–916
- Griffiths RJ, Smith MA, Roach ML, Stock JL, Stam EJ, Milici AJ et al (1997) Collagen-induced arthritis is reduced in 5-lipoxygenase-activating protein-deficient mice. *J Exp Med* 185:1123–1129
- Gupta RA, Dubois RN (2001) Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. *Nature Rev Cancer* 1:11–21
- Haklar G, Sayin-Özveri E, Yüksel M, Aktan AÖ, Yalçın AS (2001) Different kinds of reactive oxygen and nitrogen species were detected in colon and breast tumors. *Cancer Lett* 165(2):219–224
- Hammad H, de Heer HJ, Soullie T, Hoogsteden HC, Trottein F, Lambrecht BN (2003) Prostaglandin D<sub>2</sub> inhibits airway dendritic cell migration and function in steady state conditions by selective activation of the D prostanoid receptor 1. *J Immunol* 171:3936–3940
- Hao XP, Pretlow TG, Rao JS, Pretlow TP (2001) Inducible nitric oxide synthase (iNOS) is expressed similarly in multiple aberrant crypt foci and colorectal tumors from the same patients. *Cancer Res* 61:419–422
- Harris RE, Beebe-Donk J, Doss H, Burr D (2005) Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade. *Oncol Rep* 13:559–583
- Hawk ET, Levin B (2005) Colorectal cancer prevention. *J Clin Oncol* 23:378
- Hofseth LJ, Hussain SP, Wogan GN, Harris CC (2003) Nitric oxide in cancer and chemoprevention. *Free Radic Biol Med* 34:955–968
- Hong SH, Avis I, Vos MD, Martinez A, Treston AM, Mulshine JL (1999) Relationship of arachidonic acid metabolizing enzyme expression in epithelial cancer cell lines to the growth effect of selective biochemical inhibitors. *Cancer Res* 59:2223–2228
- Ishida T, Yoshida M, Arita M, Nishitani Y, Nishiumi S, Masuda A et al (2009) Resolvin E1, an endogenous lipid mediator derived from eicosapentaenoic acid, prevents dextran sulfate sodium-induced colitis. *Inflammation and Bowel Diseases* 16(1):87–95
- Ishida T, Yoshida M, Arita M, Nishitani Y, Nishiumi S, Masuda A, Mizuno S, Takagawa T, Morita Y, Kutsumi H, Inokuchi H, Serhan CN, Blumberg RS, Azuma T (2010) Resolvin E1, an endogenous lipid mediator derived from eicosapentaenoic acid, prevents dextran sulfate sodium-induced colitis. *Inflamm Bowel Dis* 16(1):87–95
- Jacoby RF, Marshall DJ, Newton MA, Novakovic K, Tutsch K, Cole CE et al (1996) Chemoprevention of spontaneous intestinal adenomas in APC Min mouse model by the nonsteroidal anti-inflammatory drug piroxicam. *Cancer Res* 56:710–714
- Jacoby RF, Seibert K, Cole CE, Kelloff G, Lubet RA (2000) The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the Min mouse model of adenomatous polyposis. *Cancer Res* 60:5040–5044
- Janakiram NB, Rao CV (2012) iNOS-selective inhibitors for cancer prevention: promise and progress. *Future Med. Chem.* 4(17):2193–2204
- Janakiram NB, Indranie C, Malisetty SV, Jagan P, Steele VE, Rao CV (2008) Chemoprevention of colon carcinogenesis by oleanolic acid and its analog in male F344 rats and modulation of COX-2 and apoptosis in human colon HT-29 cancer cells. *Pharm Res* 25(9):2151–2157
- Janakiram NB, Mohammed A, Rao CV (2011) Role of lipoxins, resolvins, and other bioactive lipids in colon and pancreatic cancer. *Cancer Metastasis Rev* 30(3–4):507–523

- Janakiram NB, Mohammed A, Ravillah D, Choi C-I, Zhang Y, Desai D, Amin S and Rao CV. (2013) Chemopreventive effects of PBI-Se, a selenium-containing analog of PBIT, on AOM-induced aberrant crypt foci in F344 rats. *Oncol Rep* 30(2):952–960
- Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 275(5297):218–220
- Juan ME, Alfaras I, Planas JM (2010) Determination of dihydroresveratrol in rat plasma by HPLC. *J Agric Food Chem* 58(12):7472–7475
- Juan ME, Alfaras I, Planas JM (2012) Colorectal cancer chemoprevention by trans-resveratrol. *Pharmacol Res* 65(6):584–591
- Juarez-Oropeza MA, Diaz-Zagoya JC, Rabinowitz JL (1987) In vivo and in vitro studies of hypocholesterolemic effects of diosgenin in rats. *Int J Biochem* 19(8):679–683
- Kato T, Hancock RL, Mohammadpour H, McGregor B, Manalo P, Khaiboullina S, Hall MR, Pardini L, Pardini RS (2002) Influence of omega-3 fatty acids on the growth of human colon carcinoma in nude mice. *Cancer Lett* 187(1–2):169–177
- Kawamori T, Lubet R, Steele VE, Kelloff GJ, Kaskey RB, Rao CV, Reddy BS (1999) Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. *Cancer Res* 59(3):597–601
- Kawamori T, Rao CV, Seibert K, Reddy BS (1998) Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. *Cancer Res* 58:409–412
- Kawamori T, Takahashi M, Watanabe K et al (2000) Suppression of azoxymethane-induced colonic aberrant crypt foci by a nitric oxide synthase inhibitor. *Cancer Lett* 148:33–37
- Kawamori T, Tanaka T, Hara A, Yamahara J, Mori H (1995) Modifying effects of naturally occurring products on the development of colonic aberrant crypt foci induced by azoxymethane in F344 rats. *Cancer Res* 55(6):1277–1282
- Kudo T, Narisawa T, Abo S (1980) Antitumor activity of indomethacin on methylazoxymethanol-induced large bowel tumors in rats. *Gann* 71(2):260–264
- Kune GA, Kune S, Watson LF (1988) Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. *Cancer Res* 1988 48:4399–4404. Reprinted *Int J Epidemiol* 2007 36:951–956
- Kwon CS, Sohn HY, Kim SH, Kim JH, Son KH, Lee JS, Lim JK, Kim JS (2003) Anti-obesity effect of *Dioscorea nipponica* Makino with lipase-inhibitory activity in rodents. *Biosci Biotechnol Biochem* 67(7):1451–1456
- La Vecchia C et al (1977) Aspirin and colorectal cancer. *Br J Cancer* 76:675–677
- Lagares-Garcia JA, Moore RA, Collier B, Heggere M, Diaz F, Qian F (2001) Nitric oxide synthase as a marker in colorectal carcinoma. *Am J Surg* 67:709–713
- Laine L, Wogen J, Yu H (2003) Gastrointestinal health care resource utilization with chronic use of COX-2-specific inhibitors versus traditional NSAIDs. *Gastroenterology* 125:389–395
- Lakatos PL, Lakatos L (2008) Risk for colorectal cancer in ulcerative colitis: changes, causes and management strategies. *World J Gastroenterol* 14:3937–3947
- Laybayle D, Fischer D, Vielh P, Frouhin F, Pariente A, Bories C, Duhamel O, Troussat M, Atti P (1991) Sulindac causes regression of rectal polyps in familial adenomatous polyposis. *Gastroenterology* 101:635–639
- Liu J (1995) Pharmacology of oleanolic acid and ursolic acid. *J Ethnopharmacol* 49:57–68
- Logan RF, Little J, Hawtin PG, Hardcastle JD (1993) Effect of aspirin and non-steroidal anti-inflammatory drugs on colorectal adenomas: case—control study of subjects participating in the Nottingham faecal occult blood screening programme. *Br Med J* 307:285–289
- Mahato SB, Sen S (1997) Advances in triterpenoid research, 1990–1994. *Phytochemistry* 44(7):1185–1236
- Mahmoud NN, Dannenberg AJ, Mestre J et al (1998) Aspirin prevents tumors in a murine model of familial adenomatous polyposis. *Medline Surg* 124:225–231
- Marnett LJ (1992) Aspirin and potential role of prostaglandins in colon cancer. *Cancer Res* 52:5575–5589

- Martel-Pelletier J, Lajeunesse D, Reboul P, Pelletier JP (2003) Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. *Ann Rheum Dis* 62:501–509
- Martinez ME, McPherson RS, Levin B, Annegers JF (1995) Aspirin and other non-steroidal anti-inflammatory drugs and risk of colorectal adenomatous polyps among endoscoped individuals. *Cancer Epidemiol Biomarkers Prev* 4:703–707
- McAnuff MA, Harding WW, Omoruyi FO, Jacobs H, Morrison EY, Asemota HN (2005) Hypoglycemic effects of steroidal saponinins isolated from Jamaican bitter yam, *Dioscorea polygonoides*. *Food Chem Toxicol* 43(11):1667–1672
- Meira LB, Bugni JM, Green SL et al (2008) DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice. *J Clin Invest* 118:2516–2525
- Metzger U, Meier J, Uhlenschmid G, Weihe H (1984) Influence of various prostaglandin synthesis inhibitors on DMH-induced rat colon cancer. *Dis Colon Rectum* 27:366–369
- Mohammed A, Janakiram NB, Li Q, Choi CI, Zhang Y, Steele VE, Rao CV (2011) Chemoprevention of colon and small intestinal tumorigenesis in APCMin/+ Mice by licoferone, a novel dual 5-LOX/COX inhibitor: potential implications for human colon cancer prevention. *Cancer Prev Res* 4(12):2015–2026
- Moorghen M, Ince P, Finney KJ, Sunter JP, Appleton DR, Watson AJA (1988) A protective effect of sulindac against chemically—induced primary colonic tumors in mice. *J Pathol* 156:341–347
- Moorghen M, Orde M, Finney KJ, Appleton DR, Watson AJ (1998) Sulindac enhances cell proliferation in DMH-treated mouse colonic mucosa. *Cell Prolif* 31:59–70
- Moreau M, Daminet S, Martel-Pelletier J, Fernandes J, Pelletier JP (2005) Superiority of the gastroduodenal safety profile of licoferone over rofecoxib, a COX-2 selective inhibitor, in dogs. *J Vet Pharmacol Ther* 28:81–86
- Mund RC, Pizato N, Bonatto S, Nunes EA, Vicenzi T, Tanhoffer R, de Oliveira HH, Curi R, Calder PC, Fernandes LC (2007) Decreased tumor growth in Walker 256 tumor-bearing rats chronically supplemented with fish oil involves COX-2 and PGE<sub>2</sub> reduction associated with apoptosis and increased peroxidation. *Prostaglandins Leukot Essent Fatty Acids* 76(2):113–120
- Murata T, Lin MI, Aritake K et al (2008) Role of prostaglandin D2 receptor DP as a suppressor of tumor hyper-permeability and angiogenesis in vivo. *Proc Natl Acad Sci* 105(50):20009–20014
- Muscat JE, Stellman SD, Wynder EL (1994) Non-steroidal anti-inflammatory drugs and colorectal cancer. *Cancer* 74:1847–1854
- Nalini N, Manju V, Menon VP (2006) Effect of spices on lipid metabolism in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis. *J Med Food* 9(2):237–245
- Nam KT, Oh SY, Ahn B, Kim YB, Jang DD, Yang KH et al (2004) Decreased Helicobacter pylori associated gastric carcinogenesis in mice lacking inducible nitric oxide synthase. *Gut* 20(53):1250–1255
- Narisawa T, Satoh M, Sano M, Takahashi T (1993) Inhibition of initiation and promotion on N-methylnitrosourea-induced colon carcinogenesis in rats by nonsteroidal anti-inflammatory agent indomethacin. *Carcinogenesis (London)* 14:1493–1497
- Narisawa T et al (1981) Inhibition of development of methylnitrosourea-induced rat colon tumors by indomethacin treatment. *Cancer Res* 41:1954–1957
- Nguyen AV, Martinez M, Stamos MJ, Moyer MP, Planutis K, Hope C, Holcombe RF (2009) Results of a phase I pilot clinical trial examining the effect of plant-derived resveratrol and grape powder on Wnt pathway target gene expression in colonic mucosa and colon cancer. *Cancer Manag Res* 1:25–37
- Nieto N, Torres MI, Ríos A, Gil A (2002) Dietary polyunsaturated fatty acids improve histological and biochemical alterations in rats with experimental ulcerative colitis. *J Nutr* 132:11–19
- Nussmeier NA et al (2005) Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. *N Engl J Med* 352:1081–1091



- Oh SF, Vickery TW, Serhan CN (2011) Chiral lipidomics of E-series resolvins: aspirin and the biosynthesis of novel mediators. *Biochim Biophys Acta* 1811(11):737–747
- Ohd JF, Nielsen CK, Campbell J, Landberg G, Lofberg H, Sjolander A (2003) Expression of the leukotriene D<sub>4</sub> receptor CysLT<sub>1</sub>, COX-2, colorectal adenocarcinomas. *Gastroenterology* 124:57–70
- Oshima H, Bartsch H (1994) Chronic infection and inflammatory processes as cancer risk factors: possible role of NO in carcinogenesis. *Mutat Res* 305:253–264
- Orner GA, Dashwood WM, Blum CA, Daz GD, Li Q, Dashwood RH (2003) Suppression of tumorigenesis in the Apc(Min) mouse: down-regulation of b-catenin signaling by a combination of tea plus sulindac. *Carcinogenesis* 24:263–267
- Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM (1996a) Suppression of intestinal polyposis in Apc delta 716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 87(5):803–809
- Oshima M, Dinchuk JE, Kargman SL (1996b) Suppression of intestinal polyposis in APCΔ716 knockout mice by inhibition of cyclooxygenase-2 (COX-2). *Cell* 87:803–809
- Oshima M, Murai N, Kargman S et al (2001) Chemoprevention of intestinal polyposis in the APCΔ716 mouse by rofecoxib, a specific cyclooxygenase-2 inhibitor. *Cancer Res* 61:1733–1740
- Patel KR, Brown VA, Jones DJ, Britton RG, Hemingway D, Miller AS, West KP, Booth TD, Perloff M, Crowell JA (2010) Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. *Cancer Res* 70:7392–7399
- Peleg II, Maibach HT, Brown SH, Wilcox CM (1994) Aspirin and non-steroidal anti-inflammatory drug use and the risk of subsequent colorectal cancer. *Arch Intern Med* 154:394–399
- Piyachaturawat P, Glinsukon T, Toskulkao C (1983) Acute and subacute toxicity of piperine in mice, rats and hamsters. *Toxicol Lett* 16(3–4):351–359
- Pizato N, Bonatto S, Yamazaki RK, Aikawa J, Nogata C, Mund RC, Nunes EA, Piconcelli M, Naliwaiko K, Curi R, Calder PC, Fernandes LC (2005) Ratio of n6 to n-3 fatty acids in the diet affects tumor growth and cachexia in Walker 256 tumor-bearing rats. *Nutr Cancer* 53(2):194–201
- Pollard M, Luckert PH (1980) Indomethacin treatment of rats with dimethylhydrazine-induced intestinal tumors. *Cancer Treat Rep* 64:1323–1327
- Pollard M, Luckert PH (1984) Effect of piroxicam on primary intestinal tumors induced in rats by *N*-methylnitrosourea. *Cancer Lett* 25:117–121
- Pollard M, Luckert PH (1981) Treatment of chemically-induced intestinal cancers with indomethacin. *Proc Soc Exp Biol Med* 167:161–164
- Qiao L, Kozoni V, Tsioulis GJ, Koutsos MI, Hanif R, Shiff SJ et al (1995) Selected eicosanoids increase the proliferation rate of human colon carcinoma cell lines and mouse colonocytes in vivo. *Biochim et Biophys Acta* 1258:215–223
- Rachmilewitz D, Stamler JS, Bachwich D, Karmeli F, Ackerman Z, Podolsky DK (1995) Enhanced colonic nitric oxide generation and nitric oxide synthase activity in ulcerative colitis and Crohn's disease. *Gut* 36:718–723
- Raju J, Mehta R (2009) Cancer chemopreventive and therapeutic effects of diosgenin, a food saponin. *Nutr Cancer* 61(1):27–35
- Raju J, Patlolla JM, Swamy MV, Rao CV (2004) Diosgenin, a steroid saponin of *Trigonella foenum graecum* (Fenugreek), inhibits azoxymethane-induced aberrant crypt foci formation in F344 rats and induces apoptosis in HT-29 human colon cancer cells. *Cancer Epidemiol Biomarkers Prev* 13(8):1392–1398
- Rao CV (2004) Nitric Oxide signaling in colon cancer chemoprevention. *Mut Res* 555:107–119
- Rao CV, Cooma I, Rodriguez JG, Simi B, El-Bayoumy K, Reddy BS (2000) Chemoprevention of familial adenomatous polyposis development in the APC(min) mouse model by 1, 4-phenylene bis(methylene) selenocyanate. *Carcinogenesis* 21:617–621
- Rao CV, Cooma I, Simi B, Manning PT, Connor JR, Reddy BS (2002) Chemopreventive properties of a selective inducible nitric oxide synthase inhibitor in colon carcinogenesis,

- administered alone or in combination with celecoxib, a selective cyclooxygenase-2 inhibitor. *Cancer Res* 62:165–170
- Rao CV, Kawamori T, Hamid R, Reddy BS (1999) Chemoprevention of colonic aberrant crypt foci by an inducible nitric oxide synthase—selective inhibitor. *Carcinogenesis* 20:641–644
- Rao CV, Reddy BS, Steele VE, Wang CX, Liu X, Ouyang N, Patlolla JM, Simi B, Kopelovich L, Rigas B (2006) Nitric oxide-releasing aspirin and indomethacin are potent inhibitors against colon cancer in azoxymethane-treated rats: effects on molecular targets. *Mol Cancer Ther* 6:1530–1538
- Rao CV, Reddy BS (2004) NSAIDs and chemoprevention. *Curr Cancer Drug Targets* 4:29–42
- Rao CV, Rivenson A, Simi B, Zang E, Kelloff G, Steele V, Reddy BS (1995) Chemoprevention of colon carcinogenesis by sulindac, a nonsteroidal anti-inflammatory agent. *Cancer Res* 55:1464–1472
- Reddy BS, Maruyama H, Kelloff G (1987) Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal anti-inflammatory drug, during different stages of rat colon tumor development. *Cancer Res* 47:5340–5346
- Reddy BS, Rao CV, Rivenson A, Kelloff G (1993) Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. *Carcinogenesis (London)* 14:1493–1497
- Reddy BS, Rao CV (2002) Novel approaches for colon cancer prevention by COX-2 inhibitors. *J Environ Pathol Toxicol Oncol* 21:155–164
- Rigau J, Pique JM, Rubio E, Planas R, Tarrech JM, Bordas JM (1991) Effect of long-term sulindac therapy on colonic polyposis. *Ann Intern Med* 115:952–954
- Sale S, Tunstall RG, Ruparelia KC, Potter GA, Steward WP, Gescher AJ (2005) Comparison of the effects of the chemopreventive agent resveratrol and its synthetic analog trans 3, 4, 5, 4'-tetramethoxystilbene (DMU-212) on adenoma development in the Apc(Min+) mouse and cyclooxygenase-2 in human-derived colon cancer cells. *Int J Cancer* 115(2):194–201
- Sandler RS et al (2003) A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N Engl J Med* 348:883–890
- Schleiffer R, Duranton B, Gossé F, Bergmann C, Raul F (2000) Nitric oxide synthase inhibition promotes carcinogen-induced preneoplastic changes in the colon of rats. *Nitric Oxide* 4(6):583–589
- Schneider Y, Duranton B, Gossé F, Schleiffer R, Seiler N, Raul F (2001) Resveratrol inhibits intestinal tumorigenesis and modulates host-defense-related gene expression in an animal model of human familial adenomatous polyposis. *Nutr Cancer* 39(1):102–107
- Schreinemachers DM, Everson RB (1994) Aspirin use and lung, colon, and breast cancer incidence in a prospective study. *Epidemiology* 5:138–146
- Sengottuvelan M, Nalini N (2006) Dietary supplementation of resveratrol suppresses colonic tumour incidence in 1, 2-dimethylhydrazine-treated rats by modulating biotransforming enzymes and aberrant crypt foci development. *Br J Nutr* 96(1):145–153
- Serhan CN (1997) Lipoxins and novel aspirin-triggered 15-epi-lipoxins (ATL). *Prostaglandins* 53:107–137
- Serhan CN (2002) Lipoxins and aspirin-triggered 15-epilipoxin biosynthesis: an update and role in anti-inflammation and pro-resolution. *Prostaglandins Other Lipid Mediat* 68–69:4333–4455
- Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, Marczylo TH, Morgan B, Hemingway D, Plummer SM, Pirmohamed M, Gescher AJ, Steward WP (2004) Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res* 10(20):6847–6854
- Sharma RA, McLelland HR, Hill KA, Ireson CR, Euden SA, Manson MM, Pirmohamed M, Marnett LJ, Gescher AJ, Steward WP (2001) Pharmacodynamic and pharmacokinetic study of oral Curcuma extract in patients with colorectal cancer. *Clin Cancer Res* 7:1894–1900
- Shpitz B, Giladi N, Sagiv E, Lev-Ari S, Liberman E, Kazanov D, Arber N (2006) Celecoxib and curcumin additively inhibit the growth of colorectal cancer in a rat model. *Digestion* 74(3–4):140–144
- Silverstein FE et al (2000) Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. Celecoxib long-term arthritis safety study. *JAMA* 284:1247–1255

- Skinner SA, Penney AG, O'Brien PE (1991) Sulindac inhibits the rate of growth and appearance of colon tumor in the rat *Arch Surg* 126:1094–1096
- Smallley WE, DuBois RN (1997) Colorectal cancer and nonsteroidal anti-inflammatory drugs. *Adv Pharmacol* 39:1–20
- Smith WL, DeWitt DL, Garavito RM (2000) Cyclooxygenases: structural, cellular, and molecular biology. *Ann Rev Biochem* 69:145–182
- Smith WL (1992) Prostanoid biosynthesis and mechanisms of action. *Am J Physiol* 263:F181–F191
- Solomon SD et al (2005) Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. *N Engl J Med* 352:1071–1080
- Son IS, Kim JH, Sohn HY, Son KH, Kim JS, Kwon CS (2007) Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (*Dioscorea* spp.), on high-cholesterol fed rats. *Biosci Biotechnol Biochem* 71(12):3063–3071
- Spagnesi MT, Tonelli F, Dolara P, Caderni G, Valanzano R, Anastasi A, Bianchini F (1994) Rectal proliferation and polyp occurrence in patients with familial adenomatous polyposis after sulindac treatment. *Gastroenterology* 106:362–366
- Suh N, Reddy BS, DeCastro A, Paul S, Lee HJ, Smolarek AK, So JY, Simi B, Wang CX, Janakiram NB, Steele V, Rao CV (2011) Combination of atorvastatin with sulindac or naproxen profoundly inhibits colonic adenocarcinomas by suppressing the p65/b-catenin/cyclin D1 signaling pathway in rats. *Cancer Prev Res* 4:1895–1902
- Suh O, Mettlin C, Petrelli NJ (1993) Aspirin use, cancer, and polyps of the large bowel. *Cancer* 72:1171–1177
- Swamy MV, Patlolla JM, Steele VE, Kopelovich L, Reddy BS, Rao CV (2006) Chemoprevention of familial adenomatous polyposis by low doses of atorvastatin and celecoxib given individually and in combination to APC<sup>Min</sup> mice. *Cancer Res* 66:7370–7377
- Szaleczky E, Pronai L, Nakazawa H, Tulassay Z (2000) Evidence of in vivo peroxynitrite formation in patients with colorectal carcinoma, higher plasma nitrate/nitrite levels, and lower protection against oxygen free radicals. *J Clin Gastroenterol* 30:47–51
- Tamir S, Tannenbaum SR (1996) The role of nitric oxide in carcinogenic process. *Biochim Biophys Acta* 1288:F31–F36
- Tenesa A, Dunlop MG (2009) New insights into the aetiology of colorectal cancer from genome-wide association studies. *Nat Rev Genet* 10:353–358
- Tessitore L, Davit A, Sarotto I, Caderni G (2000) Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting bax and p21 (CIP) expression. *Carcinogenesis* 21(8):1619–1622
- Thun MH, Namboodiri MM, Heath CW (1991) Aspirin use and reduced risk of fatal colon cancer. *N Engl J Med* 325:1593–1596
- Togni V, Ota CC, Folador A, Júnior OT, Aikawa J, Yamazaki RK, Freitas FA, Longo R, Martins EF, Calder PC, Curi R, Fernandes LC (2003) Cancer cachexia and tumor growth reduction in Walker 256 tumor-bearing rats supplemented with N-3 polyunsaturated fatty acids for one generation. *Nutr Cancer* 46(1):52–58
- Tong WG, Ding XZ, Talamonti MS, Bell RH, Adrian TE (2005) LTB<sub>4</sub> stimulates growth of human pancreatic cancer cells via MAPK and PI-3 kinase pathways. *Biochem Biophys Res Commun* 335:949–956
- Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, Pandher K, Lapointe JM, Saha S, Roach ML, Carter D, Thomas NA, Durtschi BA, McNeish JD, Hambor JE, Jakobsson PJ, Carty TJ, Perez JR, Audoly LP (2003) Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. *Proc Natl Acad Sci USA* 100:9044–9049
- Ukil A, Maity S, Karmakar S, Datta N, Vedasiromoni JR, Das PK (2003) Curcumin, the major component of food flavour turmeric, reduces mucosal injury in trinitrobenzene sulphonic acid-induced colitis. *Br J Pharmacol* 139:209–218
- Vanamala J, Glagolenko A, Yang P, Carroll RJ, Murphy ME, Newman RA, Ford JR, Braby LA, Chapkin RS, Turner ND, Lupton JR (2008) Dietary fish oil and pectin enhance

- colonocyte apoptosis in part through suppression of PPAR delta/PGE<sub>2</sub> and elevation of PGE<sub>3</sub>. *Carcinogenesis* 29(4):790–796
- Velayos FS, Loftus EV, Jess T, Harmsen WS, Bida J, Zinsmeister AR, Tremaine WJ, Sandborn WJ (2006) Predictive and protective factors associated with colorectal cancer in ulcerative colitis: a case-control study. *Gastroenterol* 130:1941–1949
- Waddel RW, Ganser GF, Cerise EJ, Loughry RW (1989) Sulindac for polyposis of the colon. *Am J Surg* 157:175–179
- Waldner MJ, Neurath MF (2008) Cytokines in colitis associated cancer: potential drug targets? *Inflamm Allergy Drug Targets* 7:187–194
- Walle T (2011) Bioavailability of resveratrol. *Ann NY Acad Sci* 1215:9–15
- Wan G, Kato N, Watanabe H (2000) High fat diet elevates the activity of inducible nitric oxide synthase and 1, 2 dimethyl-hydrazine induced aberrant crypt foci in colon of rats. *Oncolo Rep* 7:391–395
- Wang D, Dubois RN (2006) Review prostaglandins and cancer. *Gut* 55(1):115–122
- West NJ, Clark SK, Phillips RKS, Hutchinson JM, Leicester RJ, Belluzzi A, Hull MA (2010) Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. *Gut*. doi:10.1136/gut.2009.200642
- Westbrook AM, Wei B, Braun J, Schiestl RH (2009) Intestinal mucosal inflammation leads to systemic genotoxicity in mice. *Cancer Res* 69:4827–4834
- Winde G, Gumbinger HG, Osswald H, Kemper F, Bunte H (1993) The NSAID sulindac reverses rectal adenomas in colectomized patients with familial adenomatous polyposis: clinical results of a dose finding study on rectal sulindac administration. *Int J Colorect Dis* 8:13–17
- Xu G, Ren G, Xu X, Yuan H, Wang Z, Kang L, Yu W, Tian K (2010) Combination of curcumin and green tea catechins prevents dimethylhydrazine-induced colon carcinogenesis. *Food Chem Toxicol* 48(1):390–395. doi:10.1016/j.fct.2009.10.027. Epub 25 Oct 2009
- Xu W, Liu L, Charles IG (2002) Microencapsulated iNOS-expressing cells cause tumor suppression in mice. *FASEB J* 16:213–215
- Yagihashi N, Kasajima H, Sugai S et al (2000) Increased in situ expression of nitric oxide synthase in human colorectal cancer. *Virchows Arch* 436:109–114
- Yamada T, Hoshino M, Hayakawa T, Ohhara H, Yamada H, Nakazawa T, Inagaki T, Iida M, Ogasawara T, Uchida A, Hasegawa C, Murasaki G, Miyaji M, Hirata A, Takeuchi T (1997) Dietary diosgenin attenuates subacute intestinal inflammation associated with indomethacin in rats. *Am J Physiol* 273(2):G355–G364
- Ziegler CC, Rainwater L, Whelan J, McEntee MF (2004) Dietary resveratrol does not affect intestinal tumorigenesis in Apc(Min/+) mice. *J Nutr* 134(1):5–10