Types and Amount of Dietary Fat and Colon Cancer Risk: Prevention by Omega-3 Fatty Acid-Rich Diets

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Abstract

Colorectal cancer is the second most common malignancy in the Western world including the United States. In recent years there is a strong upward trend in colon cancer risk in Japan mainly due to Americanization of Japanese food habits. Several epidemiological studies point to a strong association between nutrient composition of the diet and cancer of the colon. The role of types of dietary fat, especially saturated fats of animal origin, n-6- and n-3-rich polyunsaturated fatty acids (PUFAs) in the etiology of colorectal cancer has become increasingly apparent. Epidemiological studies indicate a positive association between the dietary intake of saturated fat and/or animal fat and colon cancer risk and an inverse relationship between the intake of fish and fish oil rich in n-3 PUFAs and colon cancer development. Although the evidence from case-control studies and international correlational studies is not totally consistent, these inconsistencies may have arisen, at least in part, from methodological limitations. Animal model studies have unequivocally provided evidence that the colon tumor-promoting effect of dietary fat depends on its fatty acid composition and that high dietary n-3 PUFAs lacks colon tumor-promoting effect as compared to diets high in n-6 PUFAs or saturated fats. Diets rich in n-3 PUFAs inhibit colon carcinogenesis through the modulation of colonic ras-p21, cyclooxygenase-2, and inducible nitric oxide synthase activities and apoptosis. Gene expression analysis using DNA microarrays indicates that n-3 fatty acid, docosahexaenoic acid activates cyclin-dependent kinase inhibitors such as p21, p27, p57 and p19 and inactivates antiapoptotic Bcl-2 family of genes, and prostaglandin family of genes. These results suggest that decreasing the intake of n-6 PUFAs and saturated fats and increasing that of n-3 PUFAs, particularly eicosapentaenoic acid and docosahexaenoic acid has the potential to be a major component of colon cancer control.

Key words: dietary fat, colorectal cancer, omega-3 fatty acids, omega-6 fatty acids

Introduction

Cancer of the large bowel which is the fourth most common cancer in the world, is one of the leading causes of cancer death in both men and women in Western countries, including the United States where about 150,000 new cases of this cancer and 56,000 related deaths were reported for the year 2000 (1). Although Lucretius had articulated a possible role of over nutrition in the etiology of degenerative diseases as early as 50 B.C., it was not until 1950s epidemiological surveys and studies in experimental animals stimulated interest in understanding the relationship between diet, nutrition and cancer. Marked international differences in the incidence and mortality of colon cancer and increase of risk in populations migrating from low- to high-risk areas such as from Japan to United States suggest that environmental factors, specifically dietary habits rather than the genetic factors play an important role in the etiology of this cancer. This upward trend in incidences of colon cancer among Japanese immigrants in Hawaii and California compared to Japanese in Japan stimulated epidemiologists to investigate the reasons for this increase.

Although the relationship between nutrition and cancer is complex and sometimes perplexing to nutritionists, and to those who visualize carcinogenesis in terms of a specific carcinogen, it should be recognized that nutritional factors and diet may relate to cancer risk in several ways: (1) food additives, contaminants, a particular dietary component, or products formed during food preparation may act as carcinogens, co-carcinogens and/or promoters, (2) nutrient deficiencies and excesses may lead to biochemical/molecular alterations that may promote neoplastic processes, (3) changes in the intake of selected macronutrients may induce metabolic, biochemical and molecular abnormalities that enhance cancer risk, and (4) certain dietary constituents act as anticarcinogens or chemopreventives. During the last 3 decades, substantial progress has been made in understanding the relationship between dietary constituents and colon cancer risk. It is important to note that there is an upward trend in colon cancer risk...
in Japan, which cannot be attributed to genetic differences (2, 3). The dietary habits in Japan have dramatically changed since the 1960s and are rapidly becoming similar to those in the United States and Canada. Importantly, nutritional epidemiologic studies conducted in Japan point to the fact that increase in colon cancer in Japan has been attributed to Americanization of Japanese food habits (2, 3). In recent decades, diet especially fat intake has received considerable interest as a possible risk factor in the etiology of colorectal cancer.

This paper does not aim to provide a comprehensive review on dietary factors as they relate to colon cancer. The purpose of this review, however, is to provide a brief overview of epidemiological and preclinical studies on the association between the types and amount of dietary fat and colon cancer risk and to discuss the results of mechanistic studies thus far conducted on the relationship between the types of dietary fat and colon cancer risk. Future directions are also discussed regarding primary and secondary prevention of colon cancer by modification of dietary habits.

**Epidemiologic evidence**

International epidemiologic studies have provided evidence that most cancers in humans are closely associated with their lifestyles including dietary habits. Dietary factors are important determinants of colorectal cancer in different populations worldwide. Cancer statistics in Japan for 2001 published by the Foundation for Promotion of Cancer Research indicate that there is an upward trend in age-adjusted mortality rates for colon cancer from 1955 to 1999 (2). According to this report, the death rates due to colon cancer in Japanese men and women in 1955 were 2.9 and 3.0 respectively, whereas they increased to 14.7 and 9.8, respectively in men and women in 1999 (Table 1). This upward trend in death rates due to colon cancer is mainly attributable to Americanization of Japanese food habits. In addition, the report by the Foundation for Promotion of Cancer Research provided the data on the time trends in food consumption, which show increased dietary intakes of animal fat and meat and decreased consumption of whole grains from 1960 to 1999. For example, animal fat consumption in 1960 was about 25 g/day (per capita) whereas in 1999, it increased to about 58 g. Meat intake was increased from 19 g/day to 78 g/day (per capita) and grain consumption decreased from 453 g/day to 254 g/day during these years. The change in dietary habits in Japan may, in part, explain the time-trends in colon cancer mortality. In fact, meat consumption has been found to be associated with an increased risk for the development of colon adenomas in Japan (4).

Based on comparative data and case-control studies in Japan and the United States in the late 1960s, Wynder et al. (5) suggested that colon cancer risk is mainly associated with nutritional factors, especially dietary fat. This pioneering study led several ecological and case-control studies on the relationship between dietary fat and colon cancer (6–8), but the conduct and interpretation of some these studies have been complicated by inherent problems in testing dietary hypothesis because of lack of accurate method of measurement of types of dietary fat in populations being studied and sensitivity to reveal narrow but biologically significant differences among the cases and controls. In addition dietary fat may be a risk factor in the absence of factors that are protective such as high dietary fiber and fibrous foods. Also, several of these studies did not take into consideration types of dietary fat such as those high in n-3 and n-6 PUFAs. The importance of types of dietary fat differing in fatty acid composition rather than total fat cannot be discounted because several animal model studies using well-established colon cancer models strongly supported the notion that the colon tumor-promoting effect of dietary fat or lack of such effect depends on its fatty acid composition (9). A recent report by an expert panel assembled by AICR/WCRF came to a scientific consensus that evidence for an association between the intake of saturated fat and/or animal fat and colon cancer risk is very strong (8). Continuing population studies revealed that diets particularly high in total fat, especially animal fat are generally associated with increased risk of developing colon cancer whereas high dietary fish oil or fish reduces this risk (10, 11). A recent ecological study suggests that mortality data for colorectal cancer in 22 European countries, the United States, and Canada, correlate with the consumption of animal fat (10). That eating a diet rich in n-3 PUFAs may decrease the risk of colorectal cancer has been hypothesized in relation to fish and fish oil (10). Caygill et al. (11) reported an inverse correlation between fish and fish oil consumption and colorectal cancer. On the basis of epidemiological evidence, it is reasonable to suggest that diets high in saturated fat increase the risk of colorectal cancer whereas diets high in fish and fish oil reduce the risk.

**Laboratory animal studies**

Laboratory animal studies have provided convincing evidence that not only the amount but also types of dietary fat differing in fatty acid composition are important factors in determining the modulating effect of this nutrient in colon tumor development.

Table 1  Time trends in age-adjusted death rates (per 100,000/year) of colon cancer and in nutrient intakes in Japan

<table>
<thead>
<tr>
<th>Year</th>
<th>Age-adjusted death rates</th>
<th>Intake of nutrients (per capita/day) in grams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>1960</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>1965</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>1970</td>
<td>5.2</td>
<td>5.0</td>
</tr>
<tr>
<td>1975</td>
<td>7.0</td>
<td>6.0</td>
</tr>
<tr>
<td>1980</td>
<td>8.7</td>
<td>7.1</td>
</tr>
<tr>
<td>1985</td>
<td>10.8</td>
<td>8.3</td>
</tr>
<tr>
<td>1990</td>
<td>12.9</td>
<td>9.3</td>
</tr>
<tr>
<td>1995</td>
<td>14.8</td>
<td>9.9</td>
</tr>
<tr>
<td>1999</td>
<td>14.7</td>
<td>9.8</td>
</tr>
</tbody>
</table>

* Data from Cancer Statistics in Japan-2001, Foundation for Promotion of Cancer Research, Japan (2).
(8, 11–14). Pioneering studies by Nigro et al. (15) on the effect of diets containing 5 and 35% beef fat on azoxymethane (AOM)–induced intestinal tumors indicate that Sprague-Dawley rats fed the high beef fat diet during the initiation and postinitiation stage developed more intestinal tumors and more metastases in the abdominal cavity, lung and liver than did rats fed the low-fat diet. Beef fat is a rich source of saturated fat. Investigations were carried out in our laboratory to evaluate the promoting effects of diets containing 5 and 20% beef fat on colon carcinogenesis by a variety of colon specific carcinogens including 1,2-dimethylhydrazine, methyloxazoxanethanol acetate, 3,2'-dimethyl-4-amino-phenyl or methylnitrosourea (16). In these studies, semipurified diets containing 5 and 20% beef fat were fed to male F344 rats before, during, and after carcinogen treatment to study the effect of animal fat on the initiation and postinitiation stages of colon carcinogenesis. The results of these studies indicate that, irrespective of colon carcinogens used to induce colon tumors, diet containing a high amount of beef fat rich in saturated fatty acids had a greater colon tumor-promoting effect than the diet low in such fat. Additional studies conducted in our laboratory also demonstrate that male F344 rats fed the diets containing 20% lard high in saturated fatty acids or 20% corn oil rich in n-6 PUFA’s were more susceptible to 1,2-dimethylhydrazine-induced colon carcinogenesis compared with those fed the diets containing 5% lard or 5% corn oil (17). In a recent study, Chang et al. (18) reported a protective effect of dietary fish oil rich in n-3 PUFA’s against AOM-induced colon carcinogenesis in male Sprague-Dawley rats. High dietary fish oil significantly inhibited colon tumors as compared to high corn oil diet. In addition, colon tumor inhibition by fish oil diet was associated with lower levels of DNA damage in the distal colon compared with corn oil diet (19). These studies provided evidence that diets containing high amount of saturated fat of animal origin or n-6 PUFA’s had a greater colon tumor-enhancing effect than the diets low in such fatty acids whereas diets high in n-3 PUFA’s had no such enhancing effect when fed continuously during the initiation and postinitiation stage of carcinogenesis. It has been recognized that multifactorial and multistage model systems of cancer induction can be divided into two distinct treatment stages, initiation and postinitiation. Studies conducted in our laboratory to determine the effect of types of dietary fat administered during the initiation phase of colon carcinogenesis (before and during carcinogen treatment) demonstrate that high dietary lard but not high corn oil or fish oil increased colon carcinogenesis when fed during the initiation stage (20, 21).

Further studies in our laboratory have evaluated the modulating effects of high dietary corn oil and safflower oil rich in n-6 PUFA’s, olive oil high in monounsaturated fatty acid oleic acid, coconut oil high in medium-chain fatty acids such as lauric acid, and fish oil during the postinitiation stage of AOM-induced colon carcinogenesis in male F344 rats (13). Animals fed the diets containing high corn oil or safflower oil (23.5%) had a higher incidence of colon tumors than did those fed the diets low in fat (5%). By contrast, diets high in coconut oil, olive oil or menhaden fish oil had no such colon tumor-enhancing effect (Table 2). The varied effects of different types of fat on colon carcinogenesis during postinitiation stage suggest that fatty acid composition is one of the determining factors in colon tumor promotion by a dietary fat and that the influence of types and amount of dietary fat is exerted mostly during the postinitiation phase of carcinogenesis (13, 21, 22). In this connection it is interesting to note that in a phase II clinical trial of patients with colonic polyps, dietary fish oil supplements have in fact inhibited cell proliferation in the colonic mucosa (23).

Thus far, progress has been made with regard to the relationship between dietary fat intake and colon cancer risk, in that we know of the tumor-promoting effects of diets rich in n-6 PUFA’s and saturated fatty acids, and lack of such effects by n-3 PUFA’s. However, it should be recognized that among the sources of dietary fat, animal fat with its high-saturated fatty acid content is by far the most important contributor amounting to about 60% to the Western diet. Importantly, dietary fat intake in the United States and Canada and other Western countries where the colon cancer rates are high consists predominantly of a mixture of saturated, monounsaturated, and polyunsaturated fats (8, 24). A recent assay in mice demonstrated that high dietary fat simulating mixed lipid composition of the average Western-style diet produced dysplastic lesions in the colon indicative of tumorigenesis (25).

In view of the significance of mixed lipids in colon cancer and because of potential tumor-inhibitory properties of n-3 PUFA’s, we have conducted a study to examine the effects of high-fat diets that contain mixed lipids rich in saturated fatty acids and to

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Table 2 Effect of types and amount of dietary fat on azoxymethane-induced colon carcinogenesis in F344 rats

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Dietary treatment</th>
<th>Male (M) or female (F)</th>
<th>% animal with colon tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>5% corn oil</td>
<td>AOM, 20 mg/kg body wt, once, s.c.</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>23.5% corn oil</td>
<td>AOM, 20 mg/kg body wt, once, s.c.</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>5% safflower oil</td>
<td>AOM, 20 mg/kg body wt, once, s.c.</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>23.5% safflower oil</td>
<td>AOM, 20 mg/kg body wt, once, s.c.</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>5% olive oil</td>
<td>AOM, 20 mg/kg body wt, once, s.c.</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>23.5% olive oil</td>
<td>AOM, 20 mg/kg body wt, once, s.c.</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>23.5% coconut oil</td>
<td>AOM, 20 mg/kg body wt, once, s.c.</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>5% corn oil</td>
<td>AOM, 50 mg/kg body wt, once, s.c.</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>23.5% corn oil</td>
<td>AOM, 50 mg/kg body wt, once, s.c.</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>4% fish oil+1% corn oil</td>
<td>AOM, 50 mg/kg body wt, once, s.c.</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>22.5% fish oil+1% corn oil</td>
<td>AOM, 50 mg/kg body wt, once, s.c.</td>
<td>M</td>
</tr>
</tbody>
</table>

* Animals were fed the experimental diets containing various types and amount of dietary fat during the postinitiation stage of colon carcinogenesis, i.e., beginning one week after carcinogen treatment until termination of the study.
* Modified from Reddy and Maeura (13).
* Modified from Reddy and Maruyama (20).
* Significantly different from their respective high-fat diet (P<0.05).
* Significantly different from high-fat corn oil diet (P<0.05).
Table 3  Effect of type and amount of dietary fat administered during postinitiation phase of azoxymethane-induced colon tumor incidence and multiplicity*

<table>
<thead>
<tr>
<th>Dietary Treatment</th>
<th>Tumor incidence (% animals with tumors)</th>
<th>Tumor multiplicity (No. of tumors/rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23 wk</td>
<td>38 wk</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Total</td>
</tr>
<tr>
<td>5% Corn oil (LFCO)</td>
<td>50</td>
<td>63</td>
</tr>
<tr>
<td>20% Mixed lipids (HFML)</td>
<td>80†</td>
<td>100†</td>
</tr>
<tr>
<td>17% Fish oil+5% Corn oil (HFFO)</td>
<td>50</td>
<td>69</td>
</tr>
</tbody>
</table>

* Animals were fed the experimental diets containing HFML or HFFO diets beginning one day after carcinogen treatment until termination of the study at 23 and 38 weeks after carcinogen treatment.
† Includes adenomas and adenocarcinomas.
‡ Values are means±SE.
§ Significantly different from LFCO and HFFO diet groups, P<0.01.
| Significantly different from LFDCO and HFFO diet groups, P<0.0001–0.0002. |
| Significantly different from LFDCO and HFFO diet groups, P<0.001. |


compare them with the effects of fish oil during the different stages of colon carcinogenesis in male F344 rats (26). Colonic preneoplastic lesions, aberrant crypt foci (ACF), were assessed in animals fed the experimental diets for 8, 23 and 38 weeks. ACF were predominately observed in the distal colons of carcinogen-treated rats. Rats fed the high-fat mixed lipids (HFML) diet showed a significantly greater (77%) number of ACF/colon compared with those fed the low-fat, corn oil (LFCO) or high-fat fish oil (HFFO) diet at all time points. The incidence of multicrypt aberrant foci was also higher in the HFML diet group than in the HFFO or LFCO diet groups, suggesting that administration of the HFFO diet significantly inhibits the formation and growth of preneoplastic lesions in the colon, whereas the HFML diet promotes the growth of such lesions. Also, dietary HFML significantly increased colon tumor incidence and multiplicity when compared with the HFFO or LFCO diets (Table 3). Importantly, rats fed the HFML diet showed 100% incidence of colonic adenocarcinomas compared with incidences of 63 and 69% in rats fed the LFCO and HFFO diets, respectively (Table 3). Also, the multiplicity of adenocarcinomas was significantly higher in animals fed the HFML diet (about 4-fold increase) as compared to those fed the LFCO diet. Equally important, the HFFO diet containing 20% fat, (mostly in the form of fish oil) induced fewer tumors than HFML diet containing the same amount of total fat mostly from mixed lipids. This reinforces that both the type and the amount of fatty acids in the diet play a critical role in colon carcinogenesis. In general, overall evidence from laboratory animal studies is consistent with the epidemiological data.

Possible mode of action of types of dietary fat in colon carcinogenesis

With regard to the mode of action of saturated fats, and n-6 and n-3 PUFAs in colon carcinogenesis, several studies indicate that diets high in saturated fatty acids (beef tallow and lard) and n-6 PUFAs (corn oil or safflower oil) increase the concentration of colonic luminal secondary bile acids including deoxycholic acid and lithocholic acid, whereas dietary fish oil high in n-3 PUFAs had no such enhancing effect (16). Metabolic epidemiological studies demonstrated that populations who are on typical Western diet and at high risk for colon cancer excrete high levels of secondary bile acids (27, 28). Secondary bile acids have been shown to stimulate protein kinase C (PKC) is a manner similar to phorbol esters, induce cell proliferation and ornithine decarboxylase activity, a rate-limiting enzyme in polyamine biosynthesis, and to act as promoters in colon carcinogenesis (29–34). Collectively these observations suggest that colon tumor-promoters such as secondary bile acids that are modulated by types of dietary fat may be important for inducing cellular response in relation to colon tumor promotion.

There are studies to indicate that inducible nitric oxide synthase (iNOS) which is regulated primarily at the transcriptional levels is over-expressed in human colon adenomas (33, 34) and in chemically-induced colon tumors of laboratory animal models (36). Accumulating data also indicate that the over-production of NO by iNOS is critical to carcinogenesis process and induces deaminated DNA lesions, thus resulting in DNA damage (37). Both NO and peroxinitrate produced in the tissues by family of NOS also activate cyclooxygenase (COX)-2. These data clearly suggest a key role for NO in tumor initiation, promotion and progression. Studies conducted in our laboratory indicate that deoxycholic acid induces iNOS activity in intestinal cells (38). Pretreatment with specific PKC inhibitors suppressed iNOS expression suggesting that one of the mechanisms by which tumor promoters including secondary bile acids may involve an increase in expression of iNOS through activation pathway that enhances colon carcinogenesis (38). It is known that the fatty acid composition of cells is sensitive to diet. Studies conducted in our laboratory indicate that increasing levels of dietary fish oil in rats increased the omega-3 fatty acids, namely, DHA and EPA in the colonic mucosal membrane phospholipid fractions at the expense of omega-6 PUFAs such as linoleic acid and arachidonic acid suggesting the possibility that the DHA and EPA of fish oil can modulate the activity of membrane-bound enzymes by partially replacing arachidonic acid and linoleic acid in the phospholipid pool (39). Therefore, the types of dietary fat determine the fatty acid composition of colonic mucosal membrane phospholipids. It is well established that arachidonic acid and some of its metabolites including prostaglandins (PGs) play an important role in the intracellular signaling pathway associated with cell proliferation and gene expression. PGs increase cell proliferation, promote angiogenesis, and inhibit immune surveillance, all of which are involved in tumor growth.

Over-expression of COX-2 plays an important role in colon
carcinogenesis (40). Tsuji and DuBois (41) who have implicated COX-2 activity in the regulation of apoptosis of rat intestinal epithelial cells, have shown that over expression of COX-2 can lead to the suppression of apoptosis. Additionally, high intake of saturated fat and omega-6 PUFAs alters membrane phospholipid turnover, releasing membrane arachidonic acid from phospholipids, and affecting prostaglandin synthesis via COX enzyme (32, 33). Elevated levels of COX-2 have been observed in human colon tumors and chemically-induced colon tumors in rodents (42, 43). Normal adult rodent and human colon tissues express levels of COX-2 that are undetectable by Northern analysis. Recent reports have shown a link between the tumorigenic potential of APC mutations and arachidonic metabolism by observation that deletion of the COX-2 gene reduces the number of tumors in mice heterozygous for an APC (46) by more than 6 fold (44). Of some interest, additional evidence supporting a role for COX-2 comes from our studies, which show a marked reduction in colon tumors in rodents with highly selective COX-2 inhibitor, celecoxib (45). Recent studies conducted in our laboratory have provided convincing evidence that a HFML diet enhances AOM-induced expression of COX-2 and eicosanoid formation from arachidonic acid in colon tumors of rats whereas the HFFO diet inhibits the levels of COX-2 (26) In this study, administration of the HFML diet produced 472 +/- 33 pmol/min (mean +/- SE) of eicosanoids in the colon tumors, significantly higher levels than the low fat corn oil diet (380 +/- 29 pmol/min) or HFFO diet (348 +/- 28 pmol/min) indicating higher COX activity. This suggests that inhibition of eicosanoid production through the modulation of COX-2 activity may be important for ability of n-3 PUFAs to inhibit colon tumorigenesis. Also colon tumors of animals fed the HFML diet showed a nearly 50% lower apoptotic index than was observed in the colon tumors of rats fed the HFFO diet. The results of these studies which indicate that over expression of COX-2 in the tumors of animals fed the HFML diet in contrast to HFFO diet inhibits apoptosis and the consequent tumor burden support the contention that over expression of COX-2 can lead to the suppression of apoptosis. In colon tumors lowering the levels of PGs may be enough to slow growth by inhibiting proliferation and induction of apoptosis and thus tumor inhibition. A major question that remains to be answered is which signaling pathways are involved in downstream of the COX-2 enzyme. These could provide not only a key link between dietary fatty acids, eicosanoids, COX-2, and transcriptional regulation of colon carcinogenesis, but also provide additional molecular targets for colon cancer prevention strategies.

Recent studies from our laboratory have shown that high dietary n-6 PUFAs enhances activities of diverse enzymes including protein kinases that have been implicated directly or indirectly in colon tumor promotion whereas high-fat diet containing n-3 PUFAs appears to suppress the activities of these enzymes (34, 46). PUFAs may influence the activity of the EGF receptor/mitogen-activated protein kinase pathway, which could activate a number of oncogenes. It is interesting that several kinases have been shown to participate in ras-mediated growth-promoting signal transduction pathways (47). The ras-p21, a guanine nucleotide-binding 21-kDa protein product of ras genes that is anchored to the cytoplasmic face of plasma membrane, functions in the regulation of cell proliferation. Mutational versions of ras-p21 are implicated in the etiology of human colon cancer (48). It is also known that trafficking of pro-ras from cytosol to plasma membrane is facilitated by a series of closely linked post translational modifications including farnesylation, which is catalyzed by farnesyl protein transferase (FPTase). It appears that inhibition of ras farnesylation blocks membrane association of ras-p21 and prevents neoplastic transformation of cells. Studies conducted in our laboratory have provided data to indicate that high dietary n-6 PUFAs increases ras-p21 expression in colonic tumors whereas high dietary n-3 PUFAs appears to exert antitumor activity by interfering with posttranslational modification and membrane localization of ras-p21 through the modulation of FPTase activity, thus inhibiting ras-p21 function (49; Table 4). Several FPTase inhibitors have demonstrated selective antiproliferative activity against ras-transformed cells both in cell culture and laboratory animal studies.

Advances in recombinant DNA technology have greatly facilitated the identification of candidate genes involved in initiation, promotion and progression of colon cancer. Expression of PLK3 (Polo-like kinase-3) is negatively correlated with the development of certain tumors (50). Over expression of PLK3 has also been shown to induce apoptosis, an effect that correlates with incomplete cytokinesis and inhibit cell proliferation. We have shown recently that expression of PLK3 was down regulated in colon tumors of rats than in uninvolved colon mucosa (51). Colon tumors isolated from rats fed the diet containing high levels of n-6 PUFAs contained very low levels of PLK3 mRNA expression whereas the tumors from animals fed the diet containing high amounts of n-3 PUFAs did not exhibit any down-regulation of PLK3. These results correlate with colon tumor incidences by dietary n-3 and n-6 PUFAs. It would appear that modulation of apoptosis through PLK3 plays a significant role in n-3 PUFAs-induced colon tumor inhibition.

Additional studies conducted in our laboratory have demonstrated that DHA inhibits growth of CaCo-2 colon cancer cells in vitro and induces apoptosis (52). Using CaCo-2 cells, we also examined the effects of DHA on the genetic precursors of human colon cancer at the transscription level using DNA oligonucleotide arrays (52). Alterations in gene expression due to DHA treatment was observed to be in the multiple signaling pathways involved in the regulation of cell cycle regulatory genes, COX-2 target genes, Table 4 Effect of types and amount of dietary fat on expression levels of ras-p21 in azoxymethane-induced colon tumors in F344 rats

<table>
<thead>
<tr>
<th>Dietary regimen1</th>
<th>LF-FO</th>
<th>HF-FO</th>
<th>HHFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon mucosa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytosolic</td>
<td>0.7±0.4</td>
<td>0.6±0.2</td>
<td>3.1±1.2</td>
</tr>
<tr>
<td>Membrane-bound</td>
<td>11.9±3.0</td>
<td>17.2±4.5</td>
<td>5.9±2.3</td>
</tr>
<tr>
<td>Total</td>
<td>12.3±4.5</td>
<td>17.5±3.8</td>
<td>9.2±1.5</td>
</tr>
<tr>
<td>Colon tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytosolic</td>
<td>0.8±0.5</td>
<td>0.9±0.4</td>
<td>6.2±2.2</td>
</tr>
<tr>
<td>Membrane-bound</td>
<td>24.0±5.2</td>
<td>33.1±8.0</td>
<td>12.3±3.8</td>
</tr>
<tr>
<td>Total</td>
<td>25.0±6.2</td>
<td>34.5±7.5</td>
<td>17.9±6.2</td>
</tr>
</tbody>
</table>

1 Animals were fed the experimental diets containing high-fat (23.5%) corn oil diet (HF-FO) or high-fat fish oil diet (20.5% fish oil+3% corn oil; HHFO) beginning one day after carcinogen treatment until termination of the study. Colon mucosa and tumors were analyzed for ras-p21.

2 Results are expressed as nanograms of ras-p21/mg protein; values are mean±SD.

3 Significantly different from LF-FO and HH-FO diets, P<0.01–0.001.

Modified from Singh J, Hamid R, Reddy BS. (49)
lipoxigenases and peroxisome proliferators. Effects of DHA on cell cycle progression and induction of apoptosis were directly paralleled by an increase in the activation of several proapoptotic caspases, inactivation of antiapoptotic Bcl-2 family of genes and activation of cyclin-dependent kinase inhibitors such as p21, waf1/cip1 and p27. Comprehensive evaluation of several of these precursor genes and transcription factors will facilitate to determine the chemopreventive efficacy of DHA and other important n-3 PUFAs present in fish oil and thus to prevent colon cancer. Also, comprehensive evaluation of these precursor genes and transcription factors provided several simultaneously expressed biological activities, many of which suggest themselves as molecular targets for effective intervention by selective chemopreventive agents including nutritional factors.

Summary and recommendations

In conclusion, on the basis of epidemiological evidence from ecological and case-control studies, it is reasonable to suggest that diets high in saturated fats increase the risk of colorectal cancer, whereas diets high in n-3 PUFAs reduce its risk. The studies described here, both epidemiological and animal model, provide evidence for the beneficial effects of diets rich in n-3 PUFA in the prevention of colorectal cancer. Animal model studies have provided convincing evidence that colon tumor-promoting effect of dietary fat depends on its fatty acid composition suggesting that the composition of ingested dietary fatty acids is more critical to colon cancer risk than is the total amount of fat. Animal model studies also demonstrate that a Western-style diet high in mixed lipids including saturated fats of animal origin as well as high dietary n-6 PUFAs had a higher potential to promote colon tumorigenesis than ingestion of a diet on equivalent amount of fat containing n-3 PUFAs. Although the mechanisms by which diets high in saturated fats (such as those in Western diets) and n-6 PUFAs promote colon carcinogenesis are not fully known, the studies conducted thus far indicate that increased levels of colonic luminal secondary bile acids, modulation of ras-p21 activity, eicosanoid production via the influence on COX activity, and the expression of apoptosis by the types of dietary fat especially n-6 PUFAs may play a key role in colon carcinogenesis. Further studies are needed to determine the role of n-3 PUFAs on the modulation of critical genes that are involved in colon and other types of cancer.

Our results and those of others suggest that nutritional prevention has the potential to be a major component of colon cancer control, especially primary prevention in general population. For primary prevention of colorectal cancer, levels of dietary n-3 PUFAs should be consistent with the recommendations based on epidemiological studies of coronary heart disease as discussed by several nutritionists (53–57). These studies suggest that 1–2 fishmeals per week or as little as 30–35 g/day of fish throughout life decreases the risk of coronary heart disease. Although American RDA for omega-3 fatty acids does not exist, there is a possibility that consumption of adequate amount of fish in our daily meals based on above epidemiological studies may also reduce the risk of colorectal cancer. Lands et al. (54) have suggested that the ratio of n-6 PUFAs to n-3 PUFAs may be important for health. The varied risk for coronary heart diseases and several types of cancer among Japanese, Mediterranean populations, and Western Europeans may, at least in part, be explained on the basis of n-6 to n-3 PUFA ratios of 4, 6–8, and 10 in their diets, respectively (53). Based on preclinical and epidemiological studies on atherosclerosis and coronary heart disease, Okuyama et al. (57) recommended for a reduction in the intake of linoleic acid and increase in the intake of n-3 PUFAs so that a n-6/n-3 ratio of 2 could be achieved for effective prevention of atherosclerosis and related diseases. They further recommended that decreasing linoleic acid intake in Japanese population may bring the recommended n-6 and n-3 PUFA balance to the typical Japanese diet, but both increasing the intake of n-3 PUFAs and decreasing that of linoleic acid is necessary in Western countries for effective prevention of atherosclerosis and related diseases and probably diet-related cancers (56). This recommendation may well be applied for the effective primary prevention of colorectal cancer in general population. Importantly, consumption of fibrous foods, fruits and vegetables is also necessary for those in Japan and Western countries to reduce the risk of colorectal cancer.

It should be recognized that intervention with nutritional supplements and/or diet modification alone may not be sufficient for secondary prevention of colorectal cancer in high-risk patients such as those with benign colon polyps. However, intervention by diet modification as recommended to general population along with chemopreventive agents that either abolish or delay the development of those events, which begin with normal appearing tissues and progress to invasion and metastases is an ideal strategy for secondary prevention of colon cancer in these high-risk individuals. Growing knowledge about mechanisms by which food components act defines the opportunities to use specific nutritional components or combination of them along with chemopreventive agents at critical stages of initiation, promotion and progression. It is certain that colon cancer prevention will be a significant focus of research and intervention in high-risk individual by identifying molecular targets that can alter or stop the process of carcinogenesis. Clearly there is a need to initiate randomized double-blind controlled clinical trials in patients with sporadic colon polyps using the n-3 PUFA-rich diets in combination with chemopreventive agents to prevent the progression of events leading to malignant neoplasms.

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