RESEARCH COMMUNICATION

Suppression of Azoxymethane-induced Colonic Premalignant Lesion Formation by Coenzyme Q10 in Rats

Katsuhisa Sakano[1], Mami Takahashi[1], Mitsuaki Kitano[2], Takashi Sugimura[1], Keiji Wakabayashi[1]*

Abstract

Reactive oxygen species cause damage to proteins, lipids and DNA. Coenzyme Q10 (CoQ10) is a compound with mitochondrial bioenergetic functions. The reduced form of CoQ10 shows antioxidant activity. In the present study, effects of CoQ10 on development of azoxymethane (AOM)-induced aberrant crypt foci (ACF) and mucin-depleted foci (MDF) in F344 male rats were investigated. To induce ACF and MDF, 6-week old rats were given two weekly subcutaneous injections of AOM (15 mg/kg body weight) and also received a control diet or experimental diets containing CoQ10 (200 or 500 ppm) for 4 weeks, starting one day before the first dose of AOM. At 10 weeks of age, all animals were sacrificed and their colons were evaluated for numbers and sizes of ACF and MDF. Administration of 200 and 500 ppm CoQ10 resulted in reduction of ACF numbers, to 77% and 68% of the carcinogen control value, respectively. The percentages of ACF consisting of more than 4 crypts in these groups were also significantly lower than in the controls. Treatment with 500 ppm CoQ10 furthermore decreased the number of sialomucin-producing ACF and MDF per colon to 42% and 38% of the carcinogen control value without CoQ10, respectively. These results suggest that CoQ10 may be an effective chemopreventive agent against colon carcinogenesis.

Key Words: Azoxymethane - aberrant crypt foci - CoQ 10 - colon - chemoprevention

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Introduction

Colon cancer is one of the leading causes of cancer deaths in both men and women in Western countries (Jemal et al., 2003). In Japan, its incidence has been increasing, and it is now the third leading cause of cancer death. Primary cancer prevention, including chemoprevention, is therefore important. Several epidemiological studies have suggested that high consumption of fruit and vegetables, especially those containing high amounts of antioxidants, may protect people from colon cancer (Terry et al., 2001).

An increasing amount of experimental and epidemiological evidence implicates reactive oxygen species (ROS) in the pathogenesis of cancer. ROS, which increase in inflammation and in exposure to exogenous stimuli, including smoking, can cause DNA damage, oxidize lipids and proteins, and alter signal transcription pathways that enhance cancer risk. Antioxidative agents (e.g., vitamin E, vitamin C, N-acetylcysteine and other phytochemicals) that scavenge ROS can act as cancer preventive agents (Borek, 2004; Khanzode et al., 2004).

Coenzyme Q10 (CoQ10) is a well-known electron transporter in complexes I (NADH-ubiquinone oxidoreductase), II (succinate-ubiquinone oxidoreductase), and III (ubiquinone-cytochrome c oxidoreductase) of the mitochondrial respiratory chain (Lenaz et al., 1993; Lenaz et al., 1998), which is synthesized endogenously in humans and is found in virtually all aerobic organisms (Tran et al., 2001). CoQ10 is also taken in through food intake. In addition to its role as an electron carrier in the mitochondrial electron transport chain, the immunostimulating action of CoQ10 has been reported (Folkers et al., 1982, 1993). The reduced form of CoQ10 also acts as an antioxidant (Frei et al., 1990; Overvad et al., 1999).

Aberrant crypt foci (ACF) are generally considered as putative preneoplastic lesions for colon cancer in both rodents (Bird, 1987; McLellan and Bird,1988) and humans (Pretlow, 1991). However, most are hyperplastic and relatively few ACF present in the colon actually develop into tumors. Therefore, it is important to identify subgroups of lesions that may be more predictive of eventual tumorigenesis. Ochiai et al. have reported that dysplastic ACF can be detected by adding a simple decolorization process with 70% methanol after conventional 0.2%

[1]*Cancer Prevention Basic Research Project, National Cancer Center Research Institute, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045,* [2]*Pharmacology and Toxicology Group, Life Science Research Laboratories, Life Science RD Center, Kaneka Corporation, 1-8 Miyamae-machi, Takasago-cho, Takasago, Hyogo 676-8688, Japan * For Correspondence: Fax: 81-3-3543-9305; E-mail: kwakabay@gan2.ncc.go.jp*

methylene blue staining (2005). Additional markers of colon cancer risk have been suggested, based on their correlation with tumor formation and/or histological changes reflecting DNA mutations. These include ß-catenin accumulated crypts (BCAC) (Yamada et al., 2000; Mori et al., 2005), flat dysplastic ACF (Paulsen et al., 2005), sialomucin-producing ACF (Jenab et al., 2001) and mucin-depleted foci (MDF) (Caderni et al., 2003). ACF producing sialomucins have a higher rate of cell proliferation, higher degree of dysplasia, and greater distortion of the luminal opening than ACF producing sulfomucins (Jenab et al., 2001; Caderni et al., 1995; Uchida et al., 1997). Most MDF are histologically dysplastic and feature accumulation of β-catenin. In order to examine the chemopreventive activity of CoQ10 in colon carcinogenesis, in the present study, we investigated effects of CoQ10 on the development of azoxymethane (AOM)-induced sialomucin-producing ACF and MDF in addition to classical ACF.

Materials and Methods

Animals and diets

Male F344 rats, 5 weeks of age, were purchased from Charles River Japan (Atsugi Japan) and quarantined for 1 week before being randomized into six groups. Three animals each were housed in a plastic cage. The animal room was controlled at 23±2˚C, 50±10% humidity, and a 12-h light/dark cycle. Powdered AIN-76A (Bio-Serv., Frenchtown, NJ) was used as the basal diet during the experiment. CoQ10 (ubiquinone) was produced in Kaneka Corporation (Osaka, Japan). Water and basal diet or experimental diets, with addition and thorough mixing of CoQ10 at the indicated concentrations, prepared every week, were given ad libitum. Food consumption and body weights were measured weekly. CoQ10 was confirmed to be stable for at least 4 weeks at the room temperature when added to the basal diet.

Experimental protocol

A total of 36 male F344 rats were divided into six groups (Table 1); three or nine rats each in saline or AOM treated groups, respectively. At 6 weeks of age, all rats except those intended for vehicle treatment were given s.c. injections of AOM (Nard Institute, Ltd., Amagasaki, Japan) at a dose rate of 15 mg/kg body weight once a week for 2 weeks. The controls received equal volumes of normal saline (5 ml/kg body weight). Starting 1 day before the first dose of AOM, rats were fed on a control diet or experimental diets containing 200 or 500 ppm CoQ10 throughout the experiment. At 10 weeks of age, the rats were sacrificed under ether anesthesia to assess the occurrence of colonic lesions. The experimental protocols were approved by the Institutional Ethics Review Committee for Animal Experimentation.

Table 1. Effects of CoQ10 on Body Weights and Food Intake of Rats

Treatment	No. of rats	Body weight (g)[1]	Mean food consumption (g/rat/day)[1]
AOM	9	222 ± 7	12.5 ± 1.6
AOM + 200 ppm CoQ10	9	222 ± 11	12.7 ± 1.6
AOM + 500 ppm CoQ10	9	228 ± 8	13.2 ± 1.6
Saline	3	226 ± 10	12.9 ± 1.4
Saline + 200 ppm CoQ10	3	233 ± 9	13.2 ± 1.2
Saline + 500 ppm CoQ10	3	230 ± 7	12.7 ± 1.4

[1]Data are means ± SD

Determination of ACF and mucin production

All colons were removed, flushed with saline, slit open longitudinally from the cecum to the anus, placed between filter papers and fixed in 10% neutral buffered formalin for 24 h. They were then stained with 0.2% methylene blue in saline and placed, mucosal side up, on a microscope slide and observed under a microscope. ACF were recorded according to standard procedures used routinely in our laboratory (Kawamori et al., 1995). After ACF determination, methylene blue-stained colons were processed for high-iron diamine Alcian blue (HID-AB) staining of mucin as described previously (Caderni et al., 1995). The HID-AB method stains sulphomucins dark brown, while light or dark blue staining predominantly

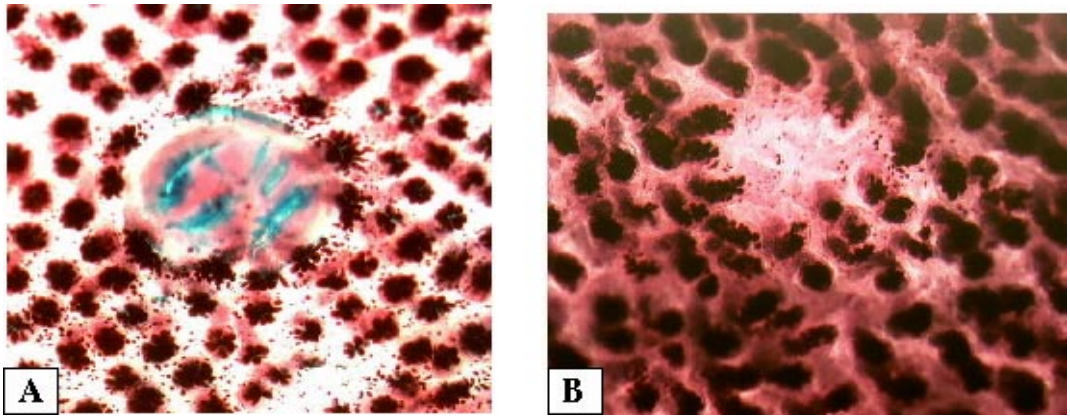

Figure. 1. Lesions identified in the colon of AOM-treated rats by HID-AB staining. (A) Appearance of a sialomucin-producing ACF (original magnification, X40). (B) Appearance of an MDF (original magnification, X40)

Table 2. Effects of CoQ10 on AOM-induced ACF formation in Male F344 rats

Treatment	Incidence (rats with ACF/total rats)	Total no. of ACF/colon[1,2]	Total no. ACs/colon[1,2]	Mean of ACs/focus[1]
AOM	9/9	278 ± 52 (100)	618 ± 82(100)	2.22 ± 0.18
AOM + 200 ppm CoQ10	9/9	213 ± 43 (77)[3]	495 ± 63 (80)[4]	2.32 ± 0.28
AOM + 500 ppm CoQ10	9/9	189 ± 41 (68)[4]	447 ± 83 (72)[4]	2.37 ± 0.17

There were no ACF in rats treated with saline. [1]Data are means ± SD [2]Data in parentheses are percentages of the carcinogen control group value. [3]Statistically different from the basal diet group at P<0.01 [4]Statistically different from the basal diet group at P<0.005

Table 3. Effects of CoQ10 on Size Distribution of AOM-induced ACF in Male F344 rats

Treatment	No. of rats	No. of ACF consisting of 1 crypt[1,2]	2 crypts[1,2]	3 crypts[1,2]	4 or more crypts[1,2]
AOM	9	69 ± 19(100)	122 ± 27(100)	60 ± 14 (100)	26 ± 17 (100)
AOM + 200 ppm CoQ10	9	50 ± 15 (72)[3]	94 ± 15 (77)[3]	48 ± 8 (80)[3]	21 ± 11 (81)[3]
AOM + 500 ppm CoQ10	9	44 ± 14 (64)[4]	83 ± 14 (68)[5]	47 ± 9 (78)[3]	15 ± 12 (58)[5]

There were no ACF in rats treated with saline. [1]Data are means ± SD [2]Data in parentheses are percentages of the carcinogen control group value. [3-5]Statistically different from the basal diet group at P<0.05, P<0.01, P<0.005.

Table 4. Effects of CoQ10 on AOM-induced Sialomucin-producing ACF Formation in Male F344 rats

Treatment	Total SP ACF/colon[1,2]	Total SP ACs/colon[1,2]	No. SP ACs/SP ACF[1]
AOM	19 ± 4(100)	46 ± 16(100)	2.4 ± 0.2
AOM + 200 ppm	10 ± 4 (53)[5]	24 ± 14 (52)[4]	2.5 ± 0.5
AOM + 500 ppm	8 ± 6 (42)[5]	19 ± 13 (41)[5]	2.1 ± 0.8

SP, sialomucin-producing. There were no SP ACF in rats treated with saline. [1]Data are means ± SD [2]Data in parentheses are percentages of the carcinogen control group value. [3-5]Statistically different from the basal diet group at P<0.05, P<0.01, P<0.005.

Table 5. Effects of CoQ10 on AOM-induced MDF Formation in Male F344 rats

Treatment	Total MDF /colon[1,2]	No. of MD crypts/colon[1,2]	No. of crypts /MDF[1]
AOM	13 ± 6(100)	28 ± 27(100)	1.9 ± 0.9
AOM + 200 ppm	9 ± 4 (69)	24 ± 28 (86)	2.6 ± 2.4
AOM + 500 ppm	5 ± 3 (38)[3]	6 ± 5 (21)[5]	1.1 ± 0.5[5]
Saline	0.7 ± 0.6	19 ± 28	29 ± 16
Saline + 200 ppm	0.3 ± 0.6	1.0 ± 1.7	3
Saline + 500 ppm	0	0	-

MD, mucin depleted. There were few MDFs in rats treated with saline. [1]Data are means ± SD [2]Data in parentheses are percentages of the carcinogen control group value. [3-5]Statistically different from the basal diet group at P<0.05, P<0.01, P<0.005.

indicates sialomucin production. White, unstained crypts are mucin-depleted. Sialomucin-producing ACF and MDF were counted.

Statistical analysis

Statistical analysis of the data on ACF, sialomucin-producing ACF and MDF was performed with the Student's t test. The results were considered statistically significant at $P < 0.05$.

Results

All animals remained healthy throughout the experimental period. Food consumption (g/day/rat) and body weight did not differ significantly among the groups (Table 1). The whole colons were stained with methylene blue and were analyzed for ACF development (Table 2). AOM caused ACF formation at a 100% incidence and the number of ACF per colon in the AOM-alone group was 278 ±52. Administration of 200 and 500 ppm CoQ10 to AOM-treated rats throughout the 4-week experimental period reduced the numbers of ACFs per colon, to 77% ($P < 0.01$) and 68% ($P < 0.005$) of the carcinogen control value, respectively. Additionally, there were significant decreases in the numbers of aberrant crypts (ACs) per colon in these groups, both in small ACF consisting of less than 3 crypts and in large ACF consisting of more than 4 crypts (Table 3). The mean number of ACs per focus in AOM-treated rats was not reduced by administration of CoQ10. No ACFs were detected in rats without AOM treatment, and fed on a basal or CoQ10-containing diet throughout the experiment. With HID-AB staining, most normal colonic crypts were observed to be producing sulphomucin. Most ACF were also producing sulphomucin, but there was a small number in which sulfomucin was decreased and sialomucin was produced (Figure 1A). In the AOM + control diet group, the number of sialomucin-producing ACF was 19±4. Administration of 200 and 500 ppm CoQ10 reduced the numbers of sialomucin-producing ACFs per colon, to 53% ($P < 0.005$) and 42% ($P < 0.005$) of the control value, respectively (Table 4). Additionally, there were significant decreases in the total numbers of sialomucin-producing aberrant crypts per colon in these groups.

MDF were identified as HID-AB-unstained crypt foci (Figure 1B). Most crypts constituting MDF were observed to be smaller than the surrounding normal crypts. In the saline-treated group, only a few MDFs were observed and a significant effect by CoQ10 was not observed. MDF formation was induced by AOM at a 100% incidence and the number of MDF per colon in the AOM-alone group was 13 ± 6 (Table 5). Overlapping ACF and MDF were not detected in this experiment. Treatment with CoQ10 at 200 and 500 ppm clearly decreased the numbers of MDFs per colon to 69% and 38% ($P < 0.005$) of the control value,

respectively. Futhermore, the total numbers of mucin-depleted crypts per colon was decreased to 86% and 25% ($P < 0.005$) of the control value. With the 500 ppm CoQ10 treatment, the mean number of mucin-depleted crypts per focus in AOM-treated rats was also significantly reduced ($P < 0.005$) (Table 5).

Discussion

The present study demonstrated that dietary administration of CoQ10 significantly suppressed formation of AOM-induced colonic lesions, including ACF, sialomucin-producing ACF and MDF in male F344 rats. Suppression was more pronounced for the dysplastic sialomucin-producing ACF and MDF than for the classical ACF. Rats fed a diet containing CoQ10 at doses of 200 and 500 ppm showed no adverse effects in food consumption and growth rate as shown in Table 1. These results suggest that CoQ10 may be an effective chemopreventive agent against colon carcinogenesis.

Consistent with our present findings, Suzuki et al. have reported that the number of invasive carcinomas and the number of lesions with epithelial dysplasia per rat in a dimethylhydrazine (DMH)-induced colonic carcinogenesis experiment were reduced to 42% and 46% of the control value, respectively, by administration of CoQ10 at a dose of 200 μg/day per rat for 23 weeks (Suzuki et al., 1986). In both cases, rats received CoQ10 before the first dose of colon carcinogen so that the preventive effects might be partly attributable to inhibition of initiation by DMH or AOM. Administration of 200 and 500 ppm CoQ10 in the present study corresponds to 2.5 and 6.3 mg/day per rat, respectively, and 570 and 1,400 mg/day per person with 50 kg of body weight, respectively. It has been reported that coenzyme Q10 is used for therapy in heart diseases and neurologic diseases at 100-1,200 mg/day in humans (Bonakdar et al., 2005). In Japan, use of 30 mg/person/day of CoQ10 is approved for therapy of congestive heart failure.

It has been reported that antioxidants, including carotenoids and tocopherols, can inhibit AOM-induced rat colonic ACF formation (Exon et al., 2004; Raju et al., 2005). CoQ10 is a free radical scavenger, and its antioxidative activity and membrane-stabilizing properties help to protect against DNA and protein damages. The reduced form of CoQ10 inhibits lipid peroxidation with an efficiency similar to that of a-tocopherol, which is considered to be a lipid-soluble antioxidant in humans (Frei et al., 1990). CoQ10 levels are reported to be significantly lower in cancer patients than in healthy controls (Folkers et al., 1997). Moreover, there is a case report that indicates some effectiveness of breast cancer therapy with CoQ10 (Lockwood et al., 1995). CoQ10 is known to enhance or otherwise modulate the immune system. Levels of immunoglobulin G in serum of patients treated with CoQ10 have been found to be increased (Folkers et al., 1982). Because immunopotentiators have been shown to reduce invasive carcinomas in the rat colon (Suzuki et al., 1986), immunostimulation may participate in inhibition of carcinogenesis.

Many studies have addressed the role of antioxidants such as vitamins A, C, and E in protection against cancers and cardiovascular diseases (Mayne, 2003). However, so-called "antioxidants" can act as prooxidants in some circumstances (Halliwell, 1999), leading to enhanced tumor development (Schwartz, 1996). Intervention trials in the USA and Finland, in fact, failed to confirm effective chemoprevention with antioxidants (Omenn et al. 1996; The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). The incidence of lung cancer in male smokers was unexpectedly increased by ß-carotene supplementation. Remarkably, a recent meta-analysis indicated that supplements of ß-carotene and vitamin A, and those of ß-carotene and vitamin E may increase mortality from gastrointestinal cancers (Bjelakovic et al., 2004). Furthermore, Stanner et al. have stressed that intervention trials have failed to show any consistent benefit from the use of anti-oxidant supplements in terms of cardiovascular disease or cancer risk, with some trials even suggesting possible harm in certain subgroups (Stanner et al., 2004). Because CoQ10 is a lipid-soluble antioxidant, as are ß-carotene and vitamin E, it is necessary to avoid an excessive intake of CoQ10.

In the present study, the numbers of ACF and MDF were decreased by the addition of CoQ10, and the size of MDFs was also reduced by addition of 500 ppm CoQ10. It is known that oxidative stress is an important factor in cancer promotion as well as initiation. The mechanism of the suppressive effect of CoQ10 on colonic lesions may be dependent on its antioxidative activity. Other mechanisms such as modulation of the immune system could also be involved in the preventive effect. In conclusion, the present study suggests that CoQ10 may be an effective chemopreventive agent against colon carcinogenesis. As deficiency of CoQ10 in humans occurs with age, its use as a supplement might be recommended for aging or chronic diseases, including cancer (Crane, 2001). Therefore, further chemopreventive studies, including long-term experiments with different doses of CoQ10, are warranted.

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References

Bird RP (1987). Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. *Cancer Lett*, **37**, 147-51.

Bjelakovic G, Nikolova D, Simonetti RG, et al (2004). Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis. *Lancet*, **364**, 1219-28.

Bonakdar RA, Guarneri E (2005). Coenzyme Q10. *Am Fam Physician*, **72**, 1065-70.

Borek C (2004). Dietary antioxidants and human cancer. *Integr Cancer Ther*, **3**, 333-41.

Caderni G, Femia AP, Giannini A, et al (2003). Identification of mucin-depleted foci in the unsectioned colon of azoxymethane-treated rats: correlation with carcinogenesis. *Cancer Res*, **63**, 2388-92.

Caderni G, Giannini A, Lancioni L, et al (1995). Characterisation of aberrant crypt foci in carcinogen-treated rats: association with intestinal carcinogenesis. *Br J Cancer*, **71**, 763-9.

Crane FL (2001). Biochemical functions of coenzyme Q10. *J Am Coll Nutr*, **20**, 591-8.

Exon JH, South EH, Taruscio TG, et al (2004). Chemopreventive effect of dietary d-alpha-tocopheryl succinate supplementation on precancer colon aberrant crypt formation and vitamin E analogue levels in young and old rats. *Nutr Cancer*, **49**, 72-80.

Folkers K, Morita M, McRee J Jr (1993). The activities of coenzyme Q10 and vitamin B6 for immune responses. *Biochem Biophys Res Commun*, **193**, 88-92.

Folkers K, Osterborg A, Nylander M, et al (1997). Activities of vitamin Q10 in animal models and a serious deficiency in patients with cancer. *Biochem Biophys Res Commun*, **234**, 296-9.

Folkers K, Shizukuishi S, Takemura K (1982). Increase in levels of IgG in serum of patients treated with coenzyme Q10. *Res Commun Chem Pathol Pharmacol*, **38**, 335-8.

Frei B, Kim MC, Ames BN (1990). Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc Natl Acad Sci U S A*, **87**, 4879-83.

Halliwell, B (1999). Oxygen and nitrogen are pro-carcinogens. Damage to DNA by reactive oxygen, chlorine and nitrogen species: measurement, mechanisms and the effects of nutrition. *Mutat Res*, **443**, 37-52.

Jemal A, Murray T, Samuels A, et al (2003). Cancer statistics, 2003. *CA Cancer J Clin*, **53**, 5-26.

Jenab M, Chen J, Thompson LU (2001). Sialomucin production in aberrant crypt foci relates to degree of dysplasia and rate of cell proliferation. *Cancer Lett*, **165**, 19-25.

Kawamori T, Tanaka T, Hara A, et al (1995). Modifying effects of naturally occurring products on the development of colonic aberrant crypt foci induced by azoxymethane in F344 rats. *Cancer Res*, **55**, 1277-82.

Khanzode SS, Muddeshwar MG, Khanzode SD, et al (2004). Antioxidant enzymes and lipid peroxidation in different stages of breast cancer. *Free Radic Res*, **38**, 81-5.

Lenaz G, Cavazzoni M, Genova ML, et al (1998). Oxidative stress, antioxidant defences and aging. *Biofactors*, **8**, 195-204.

Lenaz G, Fato R, Castelluccio C, et al (1993). The function of coenzyme Q in mitochondria. *Clin Invest*, **71**, S66-70.

Lockwood K, Moesgaard S, Yamamoto T, et al (1995). Progress on therapy of breast cancer with vitamin Q10 and the regression of metastases. *Biochem Biophys Res Commun*, **212**, 172-7.

Mayne ST (2003). Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. *J Nutr*, **133 suppl**, S933-40.

McLellan EA, Bird RP (1988). Aberrant crypts: potential preneoplastic lesions in the murine colon. *Cancer Res*, **48**, 6187-92.

Mori H, Hata K, Yamada Y, et al (2005). Significance and role of early-lesions in experimental colorectal carcinogenesis. *Chem Biol Interact*, **155**, 1-9.

Ochiai M, Watanabe M, Nakanishi M, et al (2005). Differential staining of dysplastic aberrant crypt foci in the colon facilitates prediction of carcinogenic potentials of chemicals in rats. *Cancer Lett*, **220**, 67-74.

Omenn GS, Goodman GE, Thornquist MD, et al (1996). Effects of a combination of beta-carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med*, 334, 1150-5.

Overvad K, Diamant B, Holm L, et al (1999). Coenzyme Q10 in health and disease. *Eur J Clin Nutr*, **53**, 764-70.

Paulsen JE, Loberg EM, Olstorn HB, et al. (2005) Flat dysplastic aberrant crypt foci are related to tumorigenesis in the colon of azoxymethane-treated rat. Cancer Res, 65, 121-9.

Pretlow TP, Barrow BJ, Ashton WS, et al (1991). Aberrant crypts: putative preneoplastic foci in human colonic mucosa. *Cancer Res*, **51**, 1564-7.

Raju J, Swamy MV, Cooma I, et al (2005). Low doses of beta-carotene and lutein inhibit AOM-induced rat colonic ACF formation but high doses augment ACF incidence. *Int J Cancer*, **113**, 798-802.

Schwartz JL (1996). The dual roles of nutrients as antioxidants and prooxidants: their effects on tumor cell growth. *J Nutr*, **126**, 1221S-7S.

Stanner SA, Hughes J, Kelly CN, et al (2004). A review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public Health Nutr*, **7**, 407-22.

Suzuki H, Yamamoto J, Iwata Y, et al (1986). Effects of immunostimulation with OK432, coenzyme Q10, or levamisole on dimethylhydrazine-induced colonic carcinogenesis in rats. *Jpn J Surg*, **16**, 152-5.

Terry P, Giovannucci E, Michels KB, et al (2001). Fruit, vegetables, dietary fiber, and risk of colorectal cancer. *J Natl Cancer Inst*, **93**, 525-33.

The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group (1994). The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med*, **330**, 1029-35.

Tran MT, Mitchell TM, Kennedy DT, et al (2001). Role of coenzyme Q10 in chronic heart failure, angina, and hypertension. *Pharmacotherapy*, **21**, 797-806.

Uchida K, Kado S, Onoue M, et al (1997). Relationship between the nature of mucus and crypt multiplicity in aberrant crypt foci in the rat colon. *Jpn J Cancer Res*, **88**, 807-14.

Yamada Y, Yoshimi N, Hirose Y, et al (2000). Frequent beta-catenin gene mutations and accumulations of the protein in the putative preneoplastic lesions lacking macroscopic aberrant crypt foci appearance, in rat colon carcinogenesis. *Cancer Res*, **60**, 3323-7.