A Mineral-Rich Red Algae Extract Inhibits Polyp Formation and Inflammation in the Gastrointestinal Tract of Mice on a High-Fat Diet

Muhammad N. Aslam, Tejaswi Paruchuri, Narasimharao Bhagavathula and James Varani

Integr Cancer Ther 2010 9: 93 originally published online 11 February 2010
DOI: 10.1177/1534735409360360

Integrative Cancer Therapies
9(1) 93–99

DOI: 10.1177/1534735409360360
http://ict.sagepub.com
SAGE

A Mineral-Rich Red Algae Extract Inhibits Polyp Formation and Inflammation in the Gastrointestinal Tract of Mice on a High-Fat Diet

Muhammad N. Aslam, MD,[1] Tejaswi Paruchuri, BS,[1] Narasimharao Bhagavathula, PhD,[1] and James Varani, PhD[1]

Abstract

The purpose of this study was to determine whether a mineral-rich extract derived from the red marine algae *Lithothamnion calcareum* could be used as a dietary supplement for chemoprevention against colon polyp formation. A total of 60 C57bl/6 mice were divided into 3 groups based on diet. One group received a low-fat, rodent chow diet (AIN76A). The second group received a high-fat "Western-style" diet (HFWD). The third group was fed the same HFWD with the mineral-rich extract included as a dietary supplement. Mice were maintained on the respective diets for 15 months. Autopsies were performed at the time of death or at the completion of the study. To summarize, the cumulative mortality rate was higher in mice on the HFWD during the 15-month period (55%) than in mice from the low-fat diet or the extract-supplemented high-fat diet groups (20% and 30%, respectively; $P < .05$ with respect to both). Autopsies revealed colon polyps in 20% of the animals on the HFWD and none in animals of the other 2 groups ($P < .05$). In addition to the grossly visible polyps, areas of hyperplasia in the colonic mucosa and inflammatory foci throughout the gastrointestinal tract were observed histologically in animals on the high-fat diet. Both were significantly reduced in animals on the low-fat diet and animals on the extract-supplemented HFWD. These data suggest that the mineral-rich algae extract may provide a novel approach to chemoprevention in the colon.

Keywords

colorectal cancer, chemoprevention, epithelial cell differentiation, extracellular calcium-sensing receptor, mineral-rich red algae extract, Aquamin

Introduction

Past clinical and experimental studies have demonstrated that supplementation of the diet with extracellular Ca^{2+} reduces outgrowth of premalignant polyps in colonic mucosal epithelium.[1-8] However, the effectiveness of Ca^{2+} (alone or in conjunction with vitamin D) as a chemopreventive agent is far from complete.[4,7,8] Although Ca^{2+} is known to regulate epithelial cell growth and differentiation in vitro,[9,10] our recent studies demonstrated that human colon carcinoma cell lines contain stable subpopulations of cells that are resistant to the growth-regulating activity of Ca^{2+}.[11,12] This provides an explanation at the cellular level for the failure of Ca^{2+} to completely suppress colonic polyp outgrowth in vivo. This also provides a rationale for examining other moieties as potential chemopreventative agents against colon cancer.

In the present study, we have examined a mineral-rich extract obtained from the red marine algae, *Lithothamnion calcareum*,[13] for its ability to suppress colon polyp formation in healthy mice when used as a dietary supplement over a 15-month period. It has been shown previously that differentiation of colonic epithelial cells (critical for growth control) depends on expression of the extracellular calcium-sensing receptor,[14,15] and that several trace elements found in the mineral-rich extract are more effective than Ca^{2+} itself, in upregulating the receptor.[16] This finding, along with our own recent in vitro studies showing that the extract was able to induce differentiation and suppress proliferation in colon carcinoma cells that were resistant to the growth-controlling effects of Ca^{2+} alone[17]

[1]University of Michigan Medical School, Ann Arbor, MI, USA

Corresponding Author:
Muhammad N. Aslam, Department of Pathology, University of Michigan Medical School, 1301 Catherine Street, SPC 5602, Ann Arbor, MI 48109, USA
Email: mnaslam@umich.edu

provided the rationale for the current study. Consistent with the recent in vitro observations, the present study shows that the mineral-rich extract suppresses colon polyp formation in healthy mice exposed to a high-fat diet over a 15-month period. Concomitant with suppression of colonic polyp formation is a reduction in systemic inflammatory changes that are consistently present in mice on the high-fat diet.

Materials and Methods

Red Algae Extract

The mineral-rich extract is a natural product obtained from the skeletal remains of the red marine algae, *Lithothamnion calcareum* (Pallas) J. E. Areschoug, also known as *Phymatolithon calcareum* (Pallas) W. H. Adey & D. L. McKibbin.[13] Depending on the season, a second, closely related marine algae (*Lithothamnion corallioides* Crouan) may also be present in the algae bed. The algae thrive in the cold Atlantic waters off the southwest coast of Ireland and northwest coast of Iceland. Minerals from sea water are accumulated in the algae fronds over the lifespan of the organism. Eventually, the mineral-rich fronds break off of the living organism and fall to the ocean floor, from where they are harvested. The mineralized fronds are separated from extraneous materials, sterilized, dried, and milled under ISO and HACCP certification. The mineral extract contains 12% calcium, 1% magnesium, and measurable levels of 72 other trace minerals. The extract is sold as a food supplement under the name Aquamin (GRAS 000028) and is used in various products for human consumption in Europe, Asia, Australia, and North America. The extract has been used in a recent clinical study involving subjects with osteoarthritis.[18,19]

Diets

A total of 3 diets were used in this study. These included the AIN76A rodent chow diet, a high-fat Western-style diet (HFWD), and the same HFWD supplemented with the mineral-rich algae extract. AIN76A is a routinely used low-fat rodent chow. It contains 5% fat from corn oil. The HFWD was prepared according to the formulation of Newmark et al[20] and designed to mimic the diet consumed by many individuals in Western society. It contains 20% fat from corn oil. On a per weight basis, the percentage of calories from fat in this diet is 37.8% compared with 11.5% in the AIN76A chow diet. Although sucrose is reduced in the HFWD relative to the AIN76A control diet, the overall calories provided in the HFWD are 4767 kcal% versus 3902 kcal% in the rodent chow. In addition to its high fat content, the HFWD has additional modifications. Methionine is replaced with cysteine, amounts of folic acid and choline are reduced, and most important, the Ca^{2+} level is reduced to approximately 8% of the level in normal mouse chow (5.22 g/kg). The HFWD supplemented with the algae extract is virtually identical to the unsupplemented HFWD except that the algae extract is included in the makeup at a final concentration of 62 g%. The Ca^{2+} content in the mineral-rich extract supplemented diet was 7 g/kg, consistent with the level in the Newmark et al[20] formulation. Diets were formulated and provided by Research Diets Incorporated (New Brunswick, NJ).

Treatment Protocol and Analysis

A total of 60 C57bl/6 mice (30 male and 30 female) were divided into 3 groups and maintained for 15 months on the 3 diets. Animals were closely followed throughout the maintenance phase and were weighed monthly. Animals that died during this period or were euthanized were autopsied (see below). All surviving animals were sacrificed at the end of the 15-month period and autopsied. This involved removing the entire gastrointestinal tract from the stomach to the rectum, opening it up longitudinally, and fixing the entire length of tissue in 2% buffered formalin. Then, with the aid of a dissecting microscope, the entire length of the gastrointestinal tract was examined grossly. Abnormal areas were noted and photographed. The tissue was then cut into pieces for histology. Five sections were prepared from each colon: one each from the rectum, the ascending colon, the transverse colon, the descending colon and the cecum. Three sections were also prepared from the small intestine: one each from the ileum, jejunum, and duodenum, as well as 2 from the stomach. Additional histological sections were prepared from areas with grossly visible abnormalities. The histological sections were stained with hematoxylin and eosin and evaluated microscopically. In addition, the peritoneal cavity was also carefully evaluated in each animal as were liver and spleen. Livers and spleens were weighed before fixation in 2% buffered formalin, then prepared for histology and examined at the light microscopic level. All of the procedures involving animals were reviewed and approved by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan.

Statistical Evaluation

Data were collected as the number of animals in each group with a positive finding or as averages among animals in each group. Differences among the 3 groups were evaluated statistically by χ^2 analysis or by analysis of variance followed by paired group comparisons. Differences were considered significant at the $P < .05$ level.

Results

Suppression of Colon Polyp Formation With the Mineral-Rich Extract

Healthy C57bl/6 mice were maintained for 15 months on a low-fat rodent chow diet or on a HFWD with or without the mineral-rich supplement. At the initiation of the study, all animals were 3 to 4 weeks of age and had an average weight of 16 ± 1 grams. Over the 15-month period, animals gained weight on all 3 diets. At the end of the 15-month period, mice on the AIN76A (rodent chow) diet had an average weight of 40 ± 4 grams. The weights of mice on the unsupplemented and supplemented HFWDs were 50 ± 6 and 48 ± 7 grams, respectively. This represents increases in weight of 41% and 33%, respectively, relative to weight gain of the low-fat (AIN76A) chow diet mice (both statistically different from chow diet at $P < .01$ but not statistically different from each other).

A number of animals died or were euthanized during the 15-month maintenance phase. This included 4 of 20 animals in the AIN76A group and 6 of 20 animals in the extract-supplemented HFWD group as compared with 11 of 20 animals in the unsupplemented HFWD group. Most of the animals that died before the end of the study were euthanized (in compliance with UCUCA guidelines) when they developed a severe, ulcerative dermatitis that did not respond to topical antibiotic treatment. Two additional animals were euthanized with neurological symptoms and the remainder were found dead. The differences in survival between the HFWD group and the other 2 groups were statistically significant at the $P < .05$ level by χ^2 analysis.

Animals were autopsied at the time of death or at the end of the maintenance phase. The gastrointestinal tract of each mouse was opened longitudinally and examined with the aid of a dissecting microscope. In animals on either the AIN76A control diet or the extract-supplemented HFWD, there were no detectable polyps in the colon. In animals on the HFWD without the algae supplement, 4 of the animals had detectable mucosal polyps (Table 1). In 1 of the animals, 2 polyps were observed, and in the other 3 animals, there was a single grossly detected lesion. It should be noted that although the overall rate of polyp formation in mice on the HFWD was 4 of 20 animals (20%), 10 of those animals died between months 3 and 13. None of those animals had detectable polyps. One of the animals with a polyp died at month 14 and the remaining 3 animals were from among the group of 9 mice that survived to the end of the study. Four of the polyps were in the cecum and one was in the descending colon. No lesions were detected in the small intestine or stomach. Figure 1 shows the gross and microscopic appearance of normal colonic mucosa (Figures 1A and 1D), and the appearance of 2 raised tubular polyps in the cecum of 2 different animals on the HFWD (Figures 1B, 1C, 1E, and 1F).

Table 1. Colonic Mucosal Abnormalities in C57bl/6 Mice on AIN76A Chow Diet, High-Fat "Western-Style" Diet (HFWD), and HFWD Supplemented With the Mineral-Rich Red Algae Extract[a]

	No. Positive/Total	
Treatment Group	Colon Polyps[b]	Hyperplasia/Dysplasia[c]
AIN76A (control)	0/20	4/20
HFWD	4/20[d]	12/20[e]
HFWD + extract	0/20	5/20

[a]Data are based on the entire group of animals for each treatment, that is, those that died early and those sacrificed at 15 months.
[b]Polyps: defined as raised tubular mucosal surface tumors identified grossly and confirmed histologically.
[c]Hyperplasia/dysplasia: areas of hyperplasia/dysplasia were identified microscopically but not grossly.
[d]The HFWD group was significantly different from the AIN76A control group in colon polyp formation at the $P < .05$ level by χ^2 analysis.
[e]Hyperplasia/dysplasia in the HFWD group was significantly different from that in the AIN76A control group and the HFWD + extract supplement group at the $P < .05$ level by χ^2 analysis.

The lesions are on the mucosal surface of the intestinal wall, well-circumscribed and raised. At the microscopic level, normal colonic mucosa is characterized by the presence of thin, uniform crypts with a population of well-differentiated cells at the crypt surface. In the abnormal lesions, the crypts are longer than normal and there are a variety of shapes and sizes. The crypt surface is not as smooth as it is in areas of normal mucosa.

In addition to the raised polyps identified grossly, there were areas of abnormal colonic mucosa that could be identified in microscopic sections through areas of the colon that appeared normal grossly. These areas were characterized by elongated crypts with abnormally shaped luminal openings and a serrated luminal surface (Figure 2A). Areas of histologically abnormal colonic mucosa were observed in animals from all three diet groups but were more commonly seen in animals on the HFWD than in animals from the other groups (Table 1). The abnormal glandular structures were similar to the aberrant crypts as characterized previously by other investigators.[21]

Suppression of Inflammatory Changes With the Mineral-Rich Extract

Inflammatory changes were seen in the colon of animals on the HFWD (Figures 2A and 2B). Inflammatory lesions consisted primarily of mononuclear and lymphoid cell foci. Figure 2B shows a large inflammatory nodule in a section of colon from an animal on the HFWD. Inflammatory cells fill the submucosa and extend virtually to the surface of the crypt. Foci of inflammatory cells were observed in the colons of animals in all 3 diet groups but the percentage of

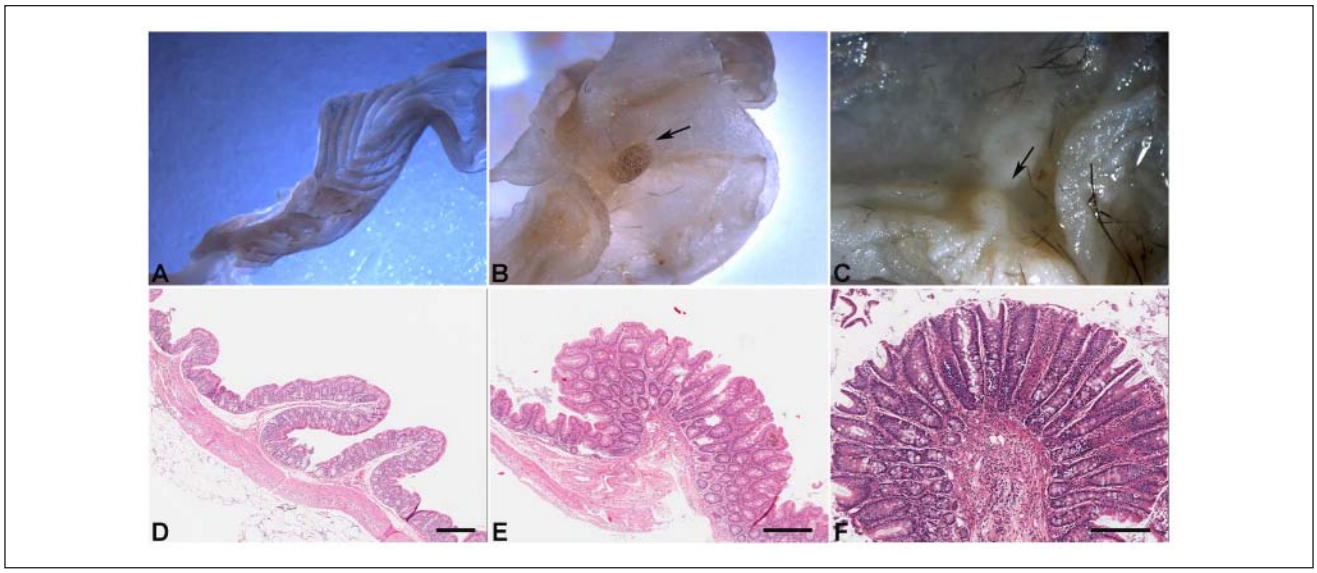

Figure 1. Appearance of normal colonic mucosa and tumors (colonic polyps) in the cecum of mice on the high-fat "Western-style" diet (HFWD)
A and D: Gross appearance (under the dissecting microscope) and histological appearance of the mucosal surface of normal colonic mucosa. The normal colonic mucosal folds are apparent at the gross level. B, C, E, and F: Gross and histological appearance of polyps identified when the cecum was opened longitudinally and examined under the dissecting microscope. (D, E, and F: hematoxylin and eosin; bar = 200 μm).

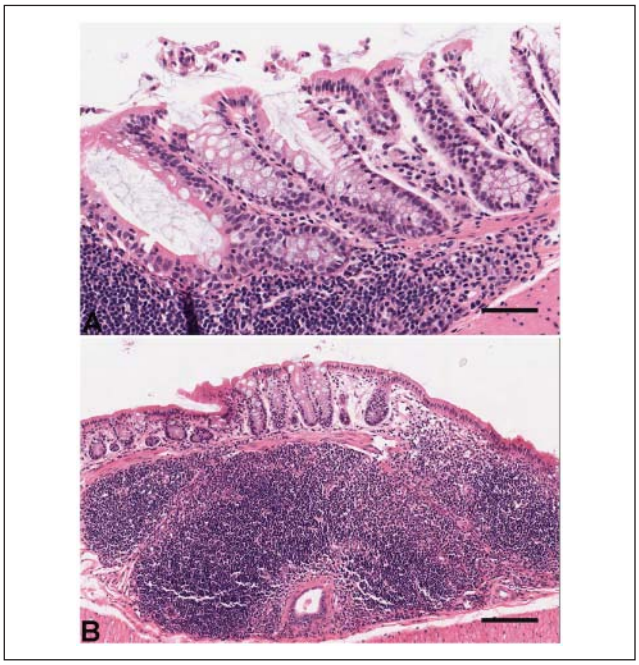

Figure 2. Histological appearance of an abnormal crypt and an inflammatory lesion in the colon.
A, Histological appearance of an abnormal crypt in the colon of a mouse on the high-fat "Western-style" diet (HFWD). The crypt is elongated and has cells with a variety of shapes and sizes. Many of the cells have a serrated border and lack the ordered differentiation seen in normal colonic mucosa. Inflammatory cells are prevalent. B, Histological appearance of an area of the colon containing an inflammatory cell nodule. Whereas the nodule itself is in the submucosa, inflammatory cells extend to the base of the mucosal epithelium. (A: hematoxylin and eosin; bar = 50 μm; B: hematoxylin and eosin; bar = 100 μm).

animals with such foci was higher in the HFWD group than in the others (Table 2). The individual foci were also larger.

In addition to the inflammatory lesions in the intestinal wall, abnormalities were also observed in the livers and spleens of mice on the HFWD. Livers were enlarged in several of the animals and average liver weight was significantly higher than in control animals (Table 2). At the histological level, areas of fatty infiltration were evident in virtually every liver. Inflammatory cells were present throughout the livers. Fibrotic changes were noted in some of the livers and 2 of the animals had large liver tumors that resembled hepatocellular carcinoma. Spleens were also enlarged in several mice on the HFWD (Table 2). Spleen enlargement was associated with an increase in extramedullary hematopoietic elements. Liver and spleen abnormalities were observed in fewer animals in both of the other 2 diet groups (Table 2).

Discussion

This report describes results from a study in which normal healthy rodents (C57bl/6 mice) were maintained for 15 months on a HFWD with and without a dietary supplement consisting of a mineral-rich extract. The HFWD was formulated to mimic the diet consumed by many individuals in Western countries.[20] Previous studies have shown that normal mice maintained on such a diet develop abnormalities in the mucosa of the large intestine, including crypt hyperplasia, colonic polyps, and occasional carcinomas.[20,22,23] In addition to containing a high content of saturated fat, the diet is

Table 2. Gastrointestinal Inflammation in C57bl/6 Mice on AIN76A Chow Diet, High-Fat "Western-Style" Diet (HFWD), and HFWD Supplemented With the Mineral-Rich Red Algae Extract[a]

	Inflammatory Nodules in Colon	Liver Enlargement		Spleen Enlargement	
Treatment Group	No. Positive/Total	No. Positive/Total	Weight (g)	No. Positive/Total	Weight (g)
AIN76A (control)	4/19	3/19	2.42 ± 0.28	4/19	0.21 ± 0.22
HFWD	10/18*	9/18*	3.69 ± 0.97*	10/18*	0.36 ± 0.27*
HFWD + extract	5/19	5/19	2.49 ± 0.61	5/19	0.29 ± 0.29

[a]Inflammatory nodules in the colon were assessed based on analysis of tissue sections from 5 different areas of the colon (rectum, descending, transverse and ascending colon, and cecum). Livers with weights >2.5 grams were considered enlarged. Spleens were considered enlarged with weights >0.3 grams. Weights are mean ± standard deviation. Statistical significance was assessed by χ^2 analysis or by analysis of variance followed by paired group comparison.
[b]Significantly different from the AIN76A control group at the $P < .05$ level.

low in a number of nutrients that are thought to protect against carcinogenesis in the gastrointestinal tract. The findings presented here substantiate earlier work demonstrating the consequences of the high-fat diet on the colonic mucosa. More important, our studies show that supplementation of the HFWD with a mineral-containing extract from the red marine algae, *L calcareum*, suppresses the outgrowth of grossly visible raised polyps and reduces microscopically visible mucosal hyperplasia.

How the mineral-rich extract prevents outgrowth of colonic polyps in the HFWD mice is not fully understood. One possibility is a direct inhibitory effect on epithelial cell proliferation. Our recent studies showed that the same mineral-rich extract induced differentiation and reduced proliferation of human colonic epithelial cells in vitro. The mineral-rich extract was equally effective with cells that were resistant to growth-regulating activity of Ca^{2+} alone as with cells that were Ca^{2+}-sensitive.[17] Our past studies have demonstrated the importance of the extracellular calcium-sensing receptor in mediating the growth-regulating effects of Ca^{2+} in the colon.[11,12,15,24,25] Other past studies have shown that many of the multivalent (lanthanide) metal ions present in the algae extract are more effective than Ca^{2+} itself in upregulating extracellular calcium-sensing receptor expression.[16,26] Based on these findings, one could hypothesize that the ability of the mineral-rich extract to directly inhibit proliferation of colonic epithelial cells underlies its effectiveness in preventing polyp outgrowth.

Alternatively, suppression of polyp outgrowth may be secondary to the concomitant inhibition of inflammatory events in the gastrointestinal tract. A high-fat diet is known to increase oxidant and inflammatory stress. In a recent study it was demonstrated that a diet rich in dairy products inhibited generation of reactive oxygen species and, concomitantly, suppressed generation of several pro-inflammatory cytokines in mice on a similarly formulated high-fat diet.[27] Ca^{2+} alone inhibited the inflammatory changes but was less effective. Consistent with these findings, several of the trace elements in the mineral-rich extract (e.g., copper, zinc, selenium, manganese, and molybdenum) are key components of antioxidant enzymes.[28] Supporting an optimal antioxidant barrier may counteract pro-oxidant and pro-inflammatory processes and thereby prevent mutagenic events that lead to a loss of growth control.

A third related possibility is that improved mucosal differentiation in the presence of the mineral-rich extract is directly related to reduced inflammation in mice on the high-fat diet. Epithelial cell differentiation is required for effective barrier formation.[29] An adequate level of Ca^{2+} is required for epithelial differentiation,[9-12] and one could imagine that in the high-fat diet (which is also low in Ca^{2+} relative to the level in normal mouse chow) failure of epithelial differentiation occurs, leading to a "leaky" barrier throughout the gastrointestinal tract. Bacteria, bacterial products, food allergens, and other toxins could then enter the submucosa and provide a constant pro-inflammatory stimulus. By promoting effective differentiation, the mineral-rich extract could counteract this effect, thereby reducing systemic inflammation and the downstream consequences of inflammation.

Mechanisms aside, it is important to ask whether the mineral-rich algae extract could be used routinely in humans as a dietary supplement under conditions needed for effective chemoprevention in the colon. The algae extract is already available as a food supplement (under the name Aquamin [GRAS 000028]) and is currently used in various products for human consumption in Europe, Asia, Australia, and North America. A recent (small) clinical study in humans has been done and no serious adverse events were observed in any of the treated subjects.[18,19] Other (nonserious) adverse events were similar in the treated group and a placebo group. Based on the positive results generated in the present study and the lack of observed detrimental effects, it would appear worthwhile to undertake a long-term comprehensive prospective study in human volunteers.

Ultimately, only such a study in humans can determine whether or not the algae extract will have effective chemopreventive activity against colon cancer.

Conclusion

In conclusion, the present study shows that a mineral-rich extract derived from the red marine algae, *L calcareum*, suppresses colon polyp formation in healthy mice exposed to a high-fat diet over a 15-month period. Concomitant with suppression of colonic polyp formation is a reduction in the systemic inflammatory changes that are seen in mice on the high-fat diet. These studies suggest a novel approach to colon cancer chemoprevention and to prevention of other consequences of a high-fat diet.

Acknowledgments

The authors would like to thank Marigot Ltd (Cork, Ireland) as the source of the mineral-rich red algae extract/supplement (Aquamin).

Declaration of Conflicting Interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research and/or authorship of this article:

United States Public Health Service (Grant No. CA140760)

References

1. Baron JA, Beach M, Mandel JS, et al. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. *N Engl J Med.* 1999;340:101-107.
2. Kampman E, Slattery ML, Caan B, Potter JD. Calcium, vitamin D, sunshine exposure, dairy products and colon cancer risk (United States). *Cancer Causes Control.* 2000;11:459-466.
3. Grau MV, Baron JA, Sandler RS, et al. Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trial. *J Natl Cancer Inst.* 2003;95:1765-1771.
4. McCullough ML, Robertson AS, Rodriguez C, et al. Calcium, vitamin D, dairy products, and risk of colorectal cancer in the Cancer Prevention Study II Nutrition Cohort (United States). *Cancer Causes Control.* 2003;14:1-12.
5. Flood A, Peters U, Chatterjee N, Lacey JV Jr, Schairer C, Schatzkin A. Calcium from diet and supplements is associated with reduced risk of colorectal cancer in a prospective cohort of women. *Cancer Epidemiol Biomarkers Prev.* 2005;14:126-132.
6. Wakai K, Hirose K, Matsuo K, et al. Dietary risk factors for colon and rectal cancers: a comparative case-control study. *J Epidemiol.* 2006;16:125-135.
7. Bostick RM, Potter JD, Sellers TA, McKenzie DR, Kushi LH, Folsom AR. Relation of calcium, vitamin D, and dairy food intake to incidence of colon cancer among older women. The Iowa Women's Health Study. *Am J Epidemiol.* 1993;137:1302-1317.
8. Kampman E, Giovannucci E, van 't Veer P, et al. Calcium, vitamin D, dairy foods, and the occurrence of colorectal adenomas among men and women in two prospective studies. *Am J Epidemiol.* 1994;139:16-29.
9. Tu CL, Oda Y, Bikle DD. Effects of a calcium receptor activator on the cellular response to calcium in human keratinocytes. *J Invest Dermatol.* 1999;113:340-345.
10. Tu CL, Oda Y, Komuves L, Bikle DD. The role of the calcium-sensing receptor in epidermal differentiation, *Cell Calcium.* 2004;35:265-273.
11. Bhagavathula N, Kelley EA, Reddy M, et al. Upregulation of calcium-sensing receptor and mitogen-activated protein kinase signalling in the regulation of growth and differentiation in colon carcinoma. *Br J Cancer.* 2005;93:1364-1371.
12. Bhagavathula N, Hanosh AW, Nerusu KC, Appelman H, Chakrabarty S, Varani J. Regulation of E-cadherin and beta-catenin by Ca^{2+} in colon carcinoma is dependent on calcium-sensing receptor expression and function. *Int J Cancer.* 2007;121:1455-1462.
13. Adey WH, McKibbin DL. Studies on the maerl species *Phymatolithon calcareum* (Pallas) nov. comb. and *Lithothamnium corallioides* Crouan in the Ria de Vigo. *Botanical Marina.* 1970;13:100-106.
14. Kallay E, Bajna E, Wrba F, Kriwanek S, Peterlik M, Cross HS. Dietary calcium and growth modulation of human colon cancer cells: role of the extracellular calcium-sensing receptor. *Cancer Detect Prevent*. 2000;24:127-136.
15. Chakrabarty S, Radjendirane V, Appelman H, Varani J. Extracellular calcium and calcium sensing receptor function in human colon carcinomas: promotion of E-cadherin expression and suppression of beta-catenin/TCF activation. *Cancer Res.* 2003;63:67-71.
16. Huang Y, Zhou Y, Castiblanco A, Yang W, Brown EM, Yang JJ. Multiple Ca^{2+} binding sites in the extracellular domain of the Ca^{2+}-sensing receptor corresponding to cooperative Ca^{2+} response. *Biochemistry.* 2009;48:388-398.
17. Aslam MN, Bhagavathula N, Paruchuri T, Hu X, Chakrabarty S, Varani J. Growth-inhibitory effects of a mineralized extract from the red algae, *Lithothamnion calcareum*, on Ca(2+)-sensitive and Ca(2+)-resistant human colon carcinoma cells. *Cancer Lett.* 2009;283:186-192.
18. Frestedt JL, Walsh M, Kuskowski MA, Zenk JL. A natural mineral supplement provides relief from knee osteoarthritis symptoms: a randomized controlled pilot trial. *Nutr J.* 2008;7:9.
19. Frestedt JL, Kuskowski MA, Zenk JL. A natural seaweed derived mineral supplement (Aquamin F) for knee arthritis: a randomized, placebo controlled pilot study. *Nutr J.* 2009;8:7.
20. Newmark HL, Yang K, Lipkin M, et al. A Western-style diet induces benign and malignant neoplasms in the colon of normal C57BI/6 mice. *Carcinogenesis.* 2001;22:1871-1875.

21. Cheng L, Lai MD. Aberrant crypt foci as microscopic precursors of colorectal cancer. *World J Gastroenterol.* 2003;9: 2642-2649.
22. Yang K, Kurihara N, Fan K, et al. Dietary induction of colonic tumors in a mouse model of sporadic colon cancer. *Cancer Res.* 2008;68:7803-7810.
23. Newmark HL, Yang K, Kurihara N, Fan K, Augenlicht LH, Lipkin M. Western-style diet-induced colonic tumors and their modulation by calcium and vitamin D in C57Bl/6 mice: a preclinical model for human sporadic colon cancer. *Carcinogenesis.* 2009;30:88-92.
24. Chakrabarty S, Wang H, Canaff L, Hendy GN, Appelman H, Varani J. Calcium sensing receptor in human colon carcinoma: interaction with Ca(2+) and 1,25-dihydroxyvitamin D(3). *Cancer Res.* 2005;65:493-498.
25. Liu G, Hu X, Varani J, Chakrabarty S. Calcium and calcium sensing receptor modulates the expression of thymidylate synthase, NAD(P)H:quinone oxidoreductase 1 and survivin in human colon carcinoma cells: promotion of cytotoxic response to mitomycin C and fluorouracil. *Mol Carcinog.* 2009;48:202-211.
26. Chang W, Shoback D. Extracellular Ca^{2+}-sensing receptors: an overview. *Cell Calcium.* 2004;35:183-196.
27. Zemel MB, Sun X. Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans. *J Nutr.* 2008;138:1047-1052.
28. Harris ED. Regulation of antioxidant enzymes. *FASEB J.* 1992;6:2675-2683.
29. Clayburgh DR, Shen L, Turner JR. A porous defense: the leaky epithelial barrier in intestinal disease. *Lab Invest.* 2004; 84:282-291.