Aberrant Crypts as a Biomarker for Colon Cancer: Evaluation of Potential Chemopreventive Agents in the Rat[1]

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Abstract

We assessed the effects of 41 potential chemopreventive agents in the F344 rat using the inhibition of carcinogen-induced aberrant crypt foci (ACF) in the colon as the measure of efficacy. ACF were induced by the carcinogen azoxymethane in F344 rats by two sequential weekly injections at a dose of 15 mg/kg. Two weeks after the last azoxymethane injection, animals were evaluated for the number of aberrant crypts detected in methylene blue-stained whole mounts of rat colon. The 41 agents were derived from a priority listing that was based on reports of chemopreventive activity in the literature and/or efficacy data from *in vitro* models of carcinogenesis. The list of agents included representative examples of phytochemicals, vitamins, minerals, inhibitors of proliferation, inducers of Phase 1 and Phase 2 metabolism systems, nonsteroidal anti-inflammatory agents, and differentiation agents. Eighteen agents were positive in the assay, significantly reducing the incidence of ACF at least in one of two doses tested. As a chemical class, the nonsteroidal anti-inflammatory drugs, which included ibuprofen, ketoprofen, piroxicam, and indomethacin, were most active; other less potent agents were arginine, butylated hydroxyanisole, curcumin, diallyl sulfide, difluoromethylornithine, 18β-glycyrrhetinic acid, indole-3-carbinol, oltipraz, purpurin, rutin, and the sodium salts of butyrate, selenite, and thiosulfate. Twenty-three agents did not inhibit ACF; included among these were several agents that promoted the development of ACF at one or both doses tested: benzyl isothiocyanate, calcium glucarate, catechin, dihydroepiandosterone, fluocinolone acetonide, folic acid, levamisole, 2-mercaptoethanesulfonic acid, nordihydroguiaretic acid, potassium glucarate, propyl gallate, β-sitosterol, sodium cromolyn, sodium molybdate, and sulfasalazine. The aberrant crypt assay demonstrates reasonable specificity and sensitivity in predicting which agents are likely to prevent colon cancer.

Introduction

For the successful implementation of cancer control in individuals at increased risk for cancer, a mechanism must exist to rapidly evaluate potential agents for future use in clinical chemoprevention trials. Traditionally, evaluation of such agents has used laboratory animal models as the standard, often with a reduction in tumor incidence as the measurement of the chemopreventive efficacy of a compound (1). Certainly for colon cancer research, one of the most well-utilized systems for efficacy testing has been the induction of tumorigenesis in the rat colon with the carcinogen, AOM.[3] The AOM model has been used very successfully to test and evaluate mechanisms of action of chemopreventive agents, some of which are also already being tested in clinical trials (2, 3). Full-length tumorigenesis experiments require both time and expense, important factors considering the ever-increasing numbers of potentially evaluable agents for chemoprevention. Attention has recently been drawn to the use of surrogate end point biomarkers for purposes of determining the usefulness of potential cancer inhibitors (4, 5).

The aberrant crypt assay capitalizes on the multistage process of colon carcinogenesis common to rats and humans. ACF are easily recognized precursors to colon cancer in carcinogen-treated rat colon visualized by the application of methylene blue staining in either fresh or fixed colonic tissue. McLellan and Bird (6) have demonstrated that ACF in rats are induced by the same carcinogens that induce cancer. That ACF contain elements of dysplasia, evidenced by alterations in enzyme activity, and express mutations in the *apc* gene and the *ras* oncogene suggest that they are part of the most commonly hypothesized pathway leading to colon cancer (7–9). Longitudinal surveillance of areas in which ACF appear suggest that these are the preferred sites for tumorigenesis, and current investigations are being directed at factors associated with the formation and progression of ACF toward colon cancer (10, 11).

With this as the background, we sought to test a list of possible chemopreventives for colon cancer identified by the Chemoprevention Branch of the NCI that specifically were active in inhibiting ACF in the AOM-treated rats. The purpose of the study was to evaluate the responsiveness of the test agents in inhibiting ACF induced by AOM during the time

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[3] The abbreviations used are: AOM, azoxymethane; ACF, aberrant crypt foci; DMBA, dimethylbenz[*a*]anthracene; NCI, National Cancer Institute; BHA, butylated hydroxyanisole; MESNA, 2-mercaptoethanesulfonic acid.

frame in which the animals were exposed to the carcinogen. Such agents, which exert their protective effect during the period of carcinogen exposure, have been termed "blocking agents" by Wattenberg (12). In this study, we report on the chemopreventive efficacy of a series of agents during this initiation phase of colon carcinogenesis.

Materials and Methods

Animals, Diets, Test Agents, and Carcinogens. Male F344 rats were purchased at 6 weeks of age from Harlan Sprague-Dawley (Wilmington, MA) and were quarantined for 5 days; then the rats were housed in standard cages and bedding in the animal facility in a 12-h light/dark cycle and 50% relative humidity with continual access to drinking water. At 7 weeks of age, all rats were fed AIN-76A diet (Dyets, Inc., Bethlehem, PA) on which they remained for the duration of the experiment. For each agent to be tested, 40 rats were randomized into groups of 10; the groups were: A, negative control (no test agent, no carcinogen); B, positive control (no test agent, with carcinogen); and C and D, treatment groups (test agent and carcinogen). The positive and negative controls were fed the standard AIN76A diet throughout the experiment's duration. The treatment groups had included in their diets either 40 or 80% of the maximum-tolerated dose of each agent; these doses were known to cause no decrement in body weight over a 5-week experimental period. This dosing protocol is common to preclinical evaluation of chemopreventive agents across other organ sites tested by the Chemoprevention Branch, NCI. The diets were fed to the rats beginning 1 week prior to injection with the carcinogen or saline, then continuously for the next 4 weeks. The test agents used in the study were procured in the highest available purity from a variety of sources. Arginine, BHA, calcium glucarate, ± catechin, curcumin, dehydroepiandosterone, diallyl sulfide, fluocinolone acetonide, folic acid, ibuprofen, indomethacin, inositol hexaphosphate, levamisole, mannitol, MESNA, piroxicam, and potassium glucarate were purchased from Sigma Chemical Co. (St. Louis, MO). Benzyl isothiocyanate, ellagic acid, indole-3-carbinol, propyl gallate, purpurin, and quercetin were purchased from Aldrich Chemical Co. (Milwaukee, WI). Nordihydroguiaretic acid was purchased from Fluka (Hauppage, NY). Phenethylisothiocyanate was purchased from Lancaster Synthesis (Wyndham, NH). Ascorbyl palmitate (Hoffman-LaRoche, Nutley, NJ), α-difluoromethylornithine (Marion Merrell Dow, Cincinnati, OH), 18β-glycyrrhetinic acid (MacAndrew and Forbes, Camden, NJ), and ketoprofen and oltipraz (Rhone-Poulenc, Vitry sur Seine, France) were provided by the Chemoprevention Branch, NCI. The positive control group and the two treatment groups were injected with the carcinogen AOM purchased from Ash Stevens, Inc. (Detroit, MI), twice weekly (weeks 2 and 3 of each experiment) at a dose level of 15 mg/kg body weight via the i.p. route. The negative control group was injected with saline in place of AOM. At the end of week 5 of each experiment, the rats were killed by CO_2 asphyxiation, and the colon was removed for evaluation of aberrant crypts. This study was approved by the Institutional Animal Care and Use committee at The University of Texas M. D. Anderson Cancer Center, protocol #11-8807832.

Aberrant Crypt Assay. The colons were removed and flushed with cold PBS and then cut open along the longitudinal median and fixed flat in 10% buffered formalin for 24 h. The method of Bird (13) was used to stain and highlight ACF. For each test agent, the number of ACF was evaluated in the 0.3% methylene blue-stained colon by a technician unaware of the treatment assignment. We scored ACF under ×40 magnification using a Nikon dissecting microscope with a fiberoptic light source to transluminate the colon.

Statistical Analysis. All data were analyzed using Sigmastat software running on a 386 PC computer. Both treatment doses were compared to the AOM-only group using one-way ANOVA. If a significant difference ($P < 0.05$) was observed, we used the Bonferroni t test as a multiple comparison test. The data were also tested for normality; if the data were not normally distributed, we used the nonparametric Kruskal-Wallis test for multiple comparisons.

Results

Table 1 summarizes the results of the efficacy of test agents in inhibiting AOM-induced foci in rat colon. The results are reported as a percentage of control. We evaluated 4–5 test agents per experimental session and screened a total of 41 compounds. The average yield of aberrant crypts combining the values for all experimental sessions for the AOM-only group was 88 ± 8 ACF/colon (mean ± SE), and the range was 77–141 ACF/colon. Statistical comparisons were made with reference to the positive control values for each experimental session. Body weights of the rats given test agents were evaluated in this study, and these were compared to the carcinogen-only group over the experimental period of 5 weeks. AOM treatment did not appreciably decrease rat body weight when compared with saline treatment over this short time frame, nor did any tested agent reduce body weights by greater than 10% during the experimental period. Of the agents tested, arginine, butylated hydroxyanisole, diallyl sulfide, difluoromethylornithine, 18β-glycyrrhetinic acid, ibuprofen, indole-3-carbinol, ketoprofen, oltipraz, and piroxicam were the most potent inhibitors of colonic ACF; all but arginine, BHA, oltipraz, and piroxicam were inhibitory in a dose-dependent fashion. Curcumin, indomethacin, purpurin, rutin, and the sodium salts of butyrate, selenite, and thiosulfate also inhibited AOM-induced ACF but only at the highest dose tested. Taken together, it is very interesting to note that, as a chemical class, the nonsteroidal anti-inflammatory drugs (*e.g.*, ibuprofen, ketoprofen, piroxicam, and to a lesser degree, indomethacin) were the most effective suppressants of aberrant crypt development in the rat colon. Although 23 compounds were nonresponsive in this assay, it is noteworthy that some agents actually promoted aberrant crypt formation. Benzyl isothiocyanate, calcium glucarate, the isomeric mixture of catechin, dehydroepiandosterone, propyl gallate, β-sitosterol, and sulfasalazine fell into this category and would be considered less likely choices for continued studies of chemoprevention in the colon because they induced a dose-dependent increase in ACF. Fluocinolone acetonide, folic acid, levamisole, MESNA, nordihydroguiaretic acid, potassium glucarate, sodium cromolyn, and sodium molybdate also increased ACF formation, but only at the higher dose tested.

Table 2 illustrates the sensitivity and specificity of inhibition of ACF as a predictor for inhibition of tumorigenesis. To compile these data, we reviewed the literature for evidence of suppression of colon tumorigenicity in the rat. The studies reviewed included rat studies where dimethylhydrazine, azoxymethane, or methylazoxymethanol were used as the initiating carcinogen since they are metabolically related (14). From these data, the calculated sensitivity of the assay in predicting inhibition of tumorigenesis was 79%, and the specificity was 80%. In summary, agents inhibiting both ACF and colon tumorigenesis were: BHA, curcumin, diallyl sulfide, di-

Table 1 Effect of test agents on AOM-induced ACF in rat colon

Agent (g/kg diet)	No. ACF/colon	(% control)[a]	Result
Arginine			
5	39 ± 3	(47)	+[b]
10	60 ± 5	(72)	+[b]
Ascorbyl palmitate			
5	89 ± 9	(114)	−
10	62 ± 9	(80)	−
Benzyl isothiocyanate			
0.5	113 ± 5	(136)	−[c]
1.0	124 ± 10	(149)	−[c]
BHA			
4.3	75 ± 10	(53)	+[b]
8.6	75 ± 5	(53)	+[b]
Calcium glucarate			
5	126 ± 18	(162)	−[c]
10	104 ± 11	(133)	−[c]
±Catechin			
2.5	105 ± 11	(135)	−[c]
5	117 ± 10	(150)	−[c]
Curcumin			
8	76 ± 9	(97)	−
16	57 ± 6	(73)	+[b]
Dehydroepiandosterone			
1	98 ± 6	(121)	−[c]
2	116 ± 9	(143)	−[c]
Diallyl sulfide			
1	111 ± 8	(79)	+[b]
2	60 ± 4	(43)	+[b]
DFMO			
2	79 ± 6	(56)	+[b]
4	45 ± 6	(29)	+[b]
Ellagic acid			
3	67 ± 11	(88)	−
6	69 ± 6	(88)	−
Fluocinolone acetonide			
0.005	108 ± 11	(130)	−[c]
0.01	97 ± 5	(117)	−
Folic acid			
2.5	136 ± 9	(164)	−[c]
5.0	81 ± 5	(98)	−
18β-Glycyrrhetinic acid			
2.5	76 ± 8	(54)	+[b]
5	52 ± 6	(37)	+[b]
Ibuprofen			
0.2	49 ± 6	(64)	+[b]
0.4	37 ± 4	(49)	+[b]
Indole-3-carbinol			
0.875	52 ± 5	(63)	+[b]
1.75	45 ± 5	(54)	+[b]
Indomethacin			
0.025	54 ± 7	(71)	−
0.05	31 ± 5	(41)	+[b]
Inositol hexaphosphate			
2.5	81 ± 7	(98)	−
5	89 ± 4	(107)	−
Ketoprofen			
0.1	72 ± 7	(81)	+[b]
0.2	50 ± 8	(56)	+[b]
Levamisole			
0.05	154 ± 18	(197)	−[c]
0.10	82 ± 8	(105)	−
Mannitol			
2.5	80 ± 5	(96)	−
5.0	81 ± 6	(98)	−
MESNA			
2.5	83 ± 6	(100)	−
5.0	113 ± 3	(136)	−[c]
Nordihydroguiaretic acid			
2	81 ± 9	(103)	−
4	120 ± 20	(154)	−[c]
Oltipraz			
0.1	57 ± 11	(63)	+[b]
0.2	70 ± 11	(78)	+[b]
Phenethyl isothiocyanate			
0.2	79 ± 9	(88)	−
0.4	98 ± 12	(108)	−
Piroxicam			
0.075	34 ± 7	(45)	+[b]
0.15	46 ± 3	(61)	+[b]
Potassium glucarate			
5	60 ± 9	(77)	−
10	129 ± 12	(165)	−[c]
Propyl gallate			
1.6	110 ± 12	(143)	−[c]
3.2	102 ± 9	(132)	−[c]
Purpurin			
1.6	84 ± 8	(95)	−
3.2	52 ± 6	(68)	+[b]
Quercetin			
15	72 ± 7	(94)	−
30	73 ± 11	(95)	−
Rutin			
15	154 ± 15	(109)	−
30	110 ± 12	(78)	+[b]
Silymarin			
2	80 ± 7	(99)	−
4	80 ± 5	(99)	−
β-Sitosterol			
3.2	138 ± 10	(165)	−[c]
6.4	137 ± 7	(165)	−[c]
Sodium butyrate			
1.25	78 ± 7	(92)	−
2.5	60 ± 4	(70)	+[b]
Sodium cromolyn			
10	78 ± 8	(103)	−
20	108 ± 15	(142)	−[c]
Sodium molybdate			
0.05	103 ± 8	(121)	−[c]
0.10	88 ± 8	(103)	−
Sodium selenite			
0.002	73 ± 8	(86)	−
0.004	60 ± 5	(71)	+[b]
Sodium thiosulfate			
2	75 ± 6	(88)	−
4	57 ± 8	(67)	+[b]
Sulfasalazine			
0.4	126 ± 14	(161)	−[c]
0.8	120 ± 9	(154)	−[c]
α-Tocopherol acetate			
1.89	88 ± 9	(109)	−[c]
3.78	70 ± 6	(86)	−
Vitamin D_3			
4500 IU	72 ± 8	(87)	−
9000 IU	76 ± 7	(92)	−

[a] Control group defined as AOM-only without test agent in diet.
[b] Significantly less than AOM-only group at $P < 0.05$.
[c] Significantly greater than AOM-only group at $P < 0.05$.

fluoromethylornithine, ibuprofen, ketoprofen, indomethacin, oltipraz, piroxicam, rutin, and selenite. Compounds shown to inhibit neoplasia but having no effect on ACF were benzylisothiocyanate, dehydroepiandrosterone, and silymarin. Only 18β-glycyrrhetinic acid and sodium butyrate were found to inhibit ACF in our study but are not known to inhibit AOM-induced colon cancer. Lastly, eight agents were found to have no effect on tumorigenesis in the literature and additionally, had no effect

Table 2 Specificity and sensitivity of the ACF assay as a predictor for colon tumor inhibition

	No. of agents	
	Inhibit tumorigenesis[a]	No effect on tumorigenesis
Inhibit ACF[b]	11	2
No effect on ACF[b]	3	8

[a] Positive inhibition of colon tumorigenesis reported in the literature (45–57).
[b] Agents tested in this study.

on AOM-induced ACF: ascorbyl palmitate, folic acid, phenethylisothiocyanate, propygallate, potassium glucarate, sulfasalazine, α-tocopherol acetate, and vitamin D_3.

Discussion

With increasing numbers of naturally occurring and synthetic compounds identified as possible inhibitors of neoplasia, it would be advantageous to economically and rapidly screen promising compounds for chemopreventive activity. In the rat, AOM-induced ACF have been suggested to be biological precursors to colon cancers (15–17). These foci share molecular and histological commonalities with tumors induced in rat colon and have been thought of as good targets for assessing the preclinical activity of chemopreventive agents (7, 18–20). In this study, we assayed 41 candidate agents to identify which were most effective in preventing the formation of ACF induced by the colon-specific carcinogen AOM. The assay, as described, provides preliminary information on efficacy for individual agents that may act as inhibitors of colon cancer and gives an initial reading as to active chemical classes of agents that may be explored in depth. The protocol we have used will not accurately assess the activity of agents that require a more chronic exposure to become effective and could possibly inaccurately classify as false negatives, agents that act in the postinitiation period only, *i.e.*, compounds that act to influence expansion and progression of aberrant crypts. To remedy this situation, we have established a postinitiation protocol in which the effects of agents acting later in the time frame of carcinogenesis are tested for effects on outgrowth and multiplicity of aberrant crypts where the potential of agents affecting ACF growth over 60 days is measured (21). We are now assaying a number of these agents that are potentially active in the postinitiation phase of carcinogenesis, targeting effects on established ACF in rat colon, and their multiplicity per dysplastic focus.

Many of the compounds shown in Table 1 have already been independently assayed for inhibition of tumorigenesis in the same animal model (22). The predictiveness of the ACF bioassay, when contrasting the effects of the tested agent in long-term colon tumor assays *versus* effects on ACF, is very good with reasonable sensitivity and specificity. Thus, most inhibitors of AOM-induced ACF are likely to be inhibitors of cancer as well. In the current study, we found the nonsteroidal anti-inflammatory drug group to be the most consistent chemical class of compounds to suppress ACF. Four of six agents thought to function by inhibition of cyclooxygenase/lipoxygenase were found to both inhibit ACF inhibition while the comparison with tumor inhibition revealed a 100% concordance for the nonsteroidal compounds. These drugs are thought to reduce the production of prostaglandins associated with advanced stages of tumorigenesis. The present study, in which the test agent is given concomitantly with the carcinogen, does not exclude, however, the possible influences on carcinogen metabolism by the oxidative pathway of prostaglandin H synthase (23). Inducers of Phase 2 drug detoxification pathways also were obviously active in the assay, as evidenced by the results for BHA, diallyl sulfide, indole-3-carbinol, and oltipraz, all of which have been shown to modulate chemically induced carcinogenesis through effects on Phase 1 or Phase 2 xenobiotic metabolism (24–30). There is increasing interest in the phenotypic expression of glutathione *S*-transferase isoforms as modulators of carcinogenesis in the human population and in compounds that may induce this critical enzyme system (26, 31, 32). Difluromethylornithine, one of the most active agents in our present study, has been shown to be a promising chemopreventive agent in previous animal studies and is already in clinical trials (33, 34). We also found arginine and 18β-glycyrrhetinic acid to strongly suppress ACF. Arginine is a naturally occurring amino acid that was reported by Perchellet *et al.* (35) to inhibit 12-*O*-tetradecanoylphorbol-13-acetate-induced mouse skin cancer; the mechanism of action is thought to be related to suppression of proliferation. Three other naturally occurring agents have been found to be active in suppressing ACF. Derived from licorice roots, 18β-glycyrrhetinic acid has been shown to have anti-inflammatory effects in animals (36). Curcumin, derived from the roots of the turmeric plant, may play a role in the reduced incidence of cancer in certain regions of the Asian subcontinent and has been shown previously to reduce aberrant crypt formation in the rat (37, 38). Rutin, a plant flavonoid, when cleaved by bacterial glycosidases in the gut, yields quercetin, a compound demonstrated by Deschner *et al.* (39, 40) to inhibit hyperproliferation in the colon of rats as well as protect against tumor development. Purpurin, a commercial dye, bears structural similarities to plant phenolic acids. Known by its trade name as Natural Red No. 8, this compound has been shown to be an effective radiosensitizer for treatment of bladder cancer in rats (41). Additionally active in inhibiting the formation of ACF were the sodium salts of butyric acid, sodium selenite, and sodium thiosulfate. Selenium salts have long been identified as potent inhibitors of chemical carcinogenesis, presumably acting through selenium-dependent glutathione peroxidase (42, 43). Sodium thiosulfate has been shown by Hochalter *et al.* (44) to inhibit chemically induced cancer.

The salient findings of this study are: (*a*) ACF, preneoplastic lesions in rat colon, are suppressed by chemopreventives agents also known to prevent tumorigenesis; (*b*) the ACF assay is rapid and efficient in identifying chemical classes of agents that have high chemopreventive potential for colon cancer; and (*c*) nonsteroidal anti-inflammatory drugs appear to be a very active class of preventive agents in this experimental system. A further strength of the ACF assay is the opportunity to intersect the biological properties that govern aberrant crypt growth and development with success or failure of a particular class of chemical chemopreventive. Ongoing studies are currently examining the postinitiation effects of these and other potential chemopreventives for effects on established ACF in an effort to suppress their growth and expansion to colonic precancers.

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References

1. Boone, C. W., Kelloff, G. J., and Malone, W. E. Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials. Cancer Res., *50:* 2–9, 1990.

2. Rao, C. V., Nayini, J., and Reddy, B. S. Effect of oltipraz 5′-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione on azoxymethane-induced biochemical changes re-

lated to early colon carcinogenesis in male F344 rats. Proc. Soc. Exp. Biol. Med., *197:* 77–84, 1991.

3. Wargovich, M. J., Allnutt, D., Palmer, C., Anaya, P., and Stephens, L. C. Inhibition of the promotional phase of azoxymethane-induced colon carcinogenesis in the F344 rat by calcium lactate: effect of simulating two human nutrient density levels. Cancer Lett., *53:* 17–25, 1990.

4. Scalmati, A., and Lipkin, M. Proliferation and differentiation biomarkers in colorectal mucosa and their application to chemoprevention studies. Environ. Health Perspect., *99:* 169–173, 1993.

5. Kulkarni, N., Zang, E., Kelloff, G., and Reddy, B. S. Effect of the chemopreventive agents piroxicam and D,L-α-difluoromethylornithine on intermediate biomarkers of colon carcinogenesis. Carcinogenesis (Lond.), *13:* 995-1000, 1992.

6. McLellan, E. A., and Bird, R. P. Aberrant crypts: potential preneoplastic lesions in the murine colon. Cancer Res., *48:* 6187–6192, 1988.

7. Vivona, A. A., Shpitz, B., Medline, A., Bruce, W. R., Hay, K., Ward, M. A., Stern, H. S., and Gallinger, S. K-ras mutations in aberrant crypt foci, adenomas and adenocarcinomas during azoxymethane-induced colon carcinogenesis. Carcinogenesis (Lond.), *14:* 1777–1781, 1993.

8. Pretlow, T. P., O'Riordan, M. A., Kolman, M. F., and Jurcisek, J. A. Colonic aberrant crypts in azoxymethane-treated F344 rats have decreased hexosaminidase activity. Am. J. Pathol., *136:* 13–16, 1990.

9. McLellan, E. A., Medline, A., Bird, R. P. Sequential analyses of the growth and morphological characteristics of aberrant crypt foci: putative preneoplastic lesions. Cancer Res., *51:* 5270–5274, 1991.

10. Berger, S. H., Haddox, M., and Wargovich, M. J. Biology of aberrant colonic crypts. Proc. Am. Assoc. Cancer Res., *35:* 100, 1994.

11. Shpitz, B., Hay, K., Medline, A., Bruce, W. R., Vivona, A., Gallinger, S., Bull, S., and Stern, H. Aberrant crypt foci: early markers of malignancy in rat colon carcinogenesis. Proc. Am. Assoc. Cancer Res., *34:* 115, 1993.

12. Wattenberg, L. W. Inhibitors of chemical carcinogenesis. Adv. Cancer Res., *26:* 197–226, 1978.

13. Bird, R. P. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. Cancer Lett., *37:* 147–151, 1987.

14. Archer, M. C., Bruce, W. R., Chan, C. C., Corpet, D. E., Medline, A., Roncucci, L., Stamp, D., and Zhang, X. M. Aberrant crypt foci and microadenoma as markers for colon cancer. Environ. Health Perspect., *98:* 195–197, 1992.

15. Sohn, O. S., Ishizaki, H., Yang, C. S., and Fiala, E. S. Metabolism of azoxymethane, methylazoxymethanol and *N*-nitrosodimethylamine by cytochrome P450IIE1. Carcinogenesis (Lond.), *12:* 127–131, 1991.

16. Pretlow, T. P., O'Riordan, M. A., Pretlow, T. G., and Stellato, T. A. Aberrant crypts in human colonic mucosa: putative preneoplastic lesions. J. Cell. Biochem. Suppl., *16G:* 55–62, 1992.

17. Roncucci, L. Early events in human colorectal carcinogenesis: aberrant crypts and microadenoma. Ital. J. Gastroenterol., *24:* 498–501, 1992.

18. Bruce, W. R. Aberrant crypt foci in the detection of colon carcinogens. Prog. Clin. Biol. Res., *347:* 129–137, 1990.

19. Lam, L. K. T., and Zhang, J. Reduction of aberrant crypt formation in the colon of CF1 mice by potential chemopreventive agents. Carcinogenesis (Lond.), *12:* 2311–2315, 1991.

20. Pereira, M. A., and Khoury, M. D. Prevention by chemopreventive agents of azoxymethane-induced foci of aberrant crypts in rat colon. Cancer Lett., *61:* 27–33, 1991.

21. Wargovich, M. J., Chen, C. D., Harris, C., Yang, E., and Velasco, M. Inhibition of aberrant crypt growth by non-steroidal anti-inflammatory agents and differentiation agents in the rat colon. Int. J. Cancer, *60:* 515–519, 1995.

22. Steele, V. E., Moon, R. C., Lubet, R. A., Grubbs, C. J., Reddy, B. S., Wargovich, M. J., McCormick, D. L., Crowell, J. A., Bagheri, D., Sigman, C. C., Boone, C. W., and Kelloff, G. J. Preclinical efficacy evaluation of potential chemopreventive agents in animal carcinogenesis models: methods and results from the NCI chemoprevention drug development program. J. Cell. Biochem., *20:* 32–54, 1994.

23. Marnett, L. J. Aspirin and the potential role of prostaglandins in colon cancer. Cancer Res., *52:* 5575–5589, 1992.

24. Hong, J. Y., Wang, Z. Y., Smith, T. J., Zhou, S., Shi, S., Pan, J., and Yang, C. S. Inhibitory effects of diallyl sulfide on the metabolism and tumorigenicity of the tobacco-specific carcinogen 4-(methylnitosamino)-1-(3-pyridyl)-1-butanone (NNK) in A/J mouse lung. Carcinogenesis (Lond.), *13:* 901–904, 1992.

25. Rao, C. V., Tokomo, K., Kelloff, G., and Reddy, B. S. Inhibition by dietary oltipraz of experimental intestinal carcinogenesis induced by azoxymethane in male F344 rats. Carcinogenesis (Lond.), *12:* 1051–1055, 1991.

26. Wattenberg, L. W., Sparnins, V. L., and Barany, G. Inhibition of *N*-nitrosodiethylamine carcinogenesis in mice by naturally occurring organosulfur compounds and monoterpenes. Cancer Res., *49:* 2689–2692, 1989.

27. Sparnins, V. L., Barany, G., and Wattenberg, L. W. Effects of organosulfur compounds from garlic and onions on benzo[*a*]pyrene-induced neoplasia and glutathione *S*-transferase activity in the mouse. Carcinogenesis (Lond.), *9:* 131–134, 1988.

28. Wattenberg, L. W. Inhibitory effects of benzyl isothiocyanate administered shortly before diethylnitrosamine or benzo[*a*]pyrene on pulmonary and forestomach neoplasia in A/J mice. Carcinogenesis (Lond.), *8:* 1971–1973, 1987.

29. deStafney, C. M., Prabhu, U. D., Sparnins, V. L., and Wattenberg, L. W. Studies related to the mechanism of 3-BHA-induced neoplasia of the rat forestomach. Food Chem. Toxicol., *24:* 1149–1157, 1986.

30. Wattenberg, L. W., and Loub, W. D. Inhibition of polycyclic hydrocarbon-induced neoplasia by naturally occurring indoles. Cancer Res., *38:* 1410–1413, 1978.

31. Clapper, M. L., Hoffman, S. J., and Tew, K. D. Glutathione-*S*-transferases in normal and malignant human colon tissue. Biochim. Biophys. Acta, *1096:* 209–216, 1991.

32. Fryer, A. A., Heagerty, A., Warwick, A., Zhao, L., Redman, C., and Strange, R. C. GSTM1 null and A, B polymorphisms at the glutathione-*S*-transferase *GSTM1* locus. Factors that confer protection and susceptibility to epithelial cancers. Proc. Am. Assoc. Cancer Res., *35:* 120, 1994.

33. Kulharni, N., Zang, E., Kelloff, G., and Reddy, B. S. Effect of the chemopreventive agents piroxicam and D,L-α-difluoromethylornithine on intermediate biomarkers of colon carcinogenesis. Carcinogenesis (Lond.), *13:* 995-1000, 1992.

34. Boone, C. W., Steele, V. E., and Kelloff, G. J. Screening for chemopreventive (anticarcinogenic) compounds in rodents. Mutat. Res., *267:* 251–255, 1992.

35. Perchellet, J. P., Posey, T. D., and Owen, M. D. Comparison of the inhibitory effects of diverse amino acids and amino acid analogs on 12-*O*-tetradecanoylphorbol-13-acetate-induced ornithine decarboxylase activity in isolated epidermal cells. Biochim. Biophys. Acta, *844:* 182–192, 1985.

36. Abe, H. Ohya, N., Yamamoto, K. F., Shibuya, T., Arichi, S., and Odashima, S. Effects of glycyrrhizin and glycyrrhetinic acid on growth and melanogenesis in cultured B16 melanoma cells. Eur. J. Cancer Clin. Oncol., *23:* 1549–1555, 1987.

37. Rao, C. V., and Reddy, B. S. Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. Carcinogenesis (Lond.), *14:* 2219–2225, 1993.

38. Huang, M. T., Deschner, E. E., Newmark, H. L., Wang, Z. Y., Ferraro, T. A., and Conney, A. H. Effect of dietary curcumin and ascorbyl palmitate on azoxymethanol-induced colonic epithelial cell proliferation and focal areas of dysplasia. Cancer Lett., *64:* 117–121, 1992.

39. Deschner, E. E., Ruperto, J. F., Wong, G. Y., and Newmark, H. L. The effect of dietary quercetin and rutin on AOM-induced acute colonic epithelial abnormalities in mice fed a high-fat diet. Nutr. Cancer, *20:* 199–204, 1993.

40. Deschner, E. E., Ruperto, J., Wong, G., and Newmark, H. L. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. Carcinogenesis (Lond.), *12:* 1193–1196, 1991.

41. Keck, R. W., Kreimer-Birnbaum, M., and Morgan, A. R. A dose response analysis of purpurin derivatives used as photosensitizers for the photodynamic treatment of transplantable FANFT induced urothelial tumors. J. Urol., *137:* 1255–1257, 1987.

42. Ip, C., and Ganther, H. E. Comparison of selenium and sulfur analogs in cancer prevention. Carcinogenesis (Lond.), *13:* 1167–1170, 1992.

43. Ip, C., Hayes, C., Budnick, R. M., and Ganther, H. E. Chemical form of selenium, critical metabolites, and cancer prevention. Cancer Res., *51:* 595–600, 1991.

44. Hochalter, J. B., Wattenberg, L. W., and Galbraith, A. R. Inhibition of β-propiolactone-induced mutagenesis and neoplasia by sodium thiosulfate. Cancer Res., *47:* 4351–4354, 1987.

45. Pereira, M. A., Barnes, L. H., Rassman, V. L., Kelloff, G. L., and Steele, V. E. Use of azoxymethane-induced foci of aberrant crypts in rat colon to identify potential cancer chemopreventive agents. Carcinogenesis (Lond.), *15:* 1049–1054, 1994.

46. Sugie, S., Okamoto, K., Okumura, A., Tanaka, T., and Mori, H. Inhibitory effects of benzyl thiocyanate and benzyl isothiocyanate on methylazoxymethanol acetate-induced intestinal carcinogenesis in rats. Carcinogenesis (Lond.), *15:* 1555–1560, 1994.

47. Rao, C. V., Rivenson, A., Simi, B., and Reddy, B. S. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer Res., *55:* 259–266, 1995.

48. Rao, C. V., Tokumo, K., Rigotty, J., Zang, E., Kelloff, G., and Reddy, B. S. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, α-difluoromethylornithine, 16α-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. Cancer Res., *51:* 4528–4534, 1991.

49. Balansky, R., Blagoeva, P., Mircheva, Z., Pozharisski, K., and de Flora, S. Effect of metabolic inhibitors, methylxanthines, antioxidants, alkali metals, and corn oil on 1,2-dimethylhydrazine carcinogenicity in rats. Anticancer Res., *12:* 933–940, 1992.

50. Rao, C. V., and Reddy, B. S. Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. Carcinogenesis (Lond.), *14:* 2219–2225, 1993.

51. Reddy, B. S., Tokomo, K., Kulkarni, N., Aligia, C., and Kelloff, G. Inhibition of colon carcinogenesis by prostaglandin synthesis inhibitors and related compounds. Carcinogenesis (Lond.), *13:* 1019–1032, 1992.

52. Gershbein, L. L. Action of dietary trypsin, pressed coffee oil, silymarin and iron salt on 1,2-dimethylhydrazine tumorigenesis by gavage. Anticancer Res., *14:* 1113–1116, 1994.

53. Deschner, E. E., Ruperto, J. F., Wong, G. Y., and Newmark, H. L. The effect of dietary quercetin and rutin on AOM-induced acute colonic epithelial abnormalities in mice fed a high-fat diet. Nutr. Cancer, *20:* 199–204, 1993.

54. Huang, M. T., Deschner, E. E., Newmark, H. L., Wang, Z. Y., Ferraro, T. A., and Conney, A. H. Effect of dietary curcumin and ascorbyl palmitate on azoxymethanol-induced colonic epithelial cell proliferation and focal areas of dysplasia. Cancer Lett., *64:* 117–121, 1992.

55. Deschner, E. E., Ruperto, J., Wong, G., and Newmark, H. L. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. Carcinogenesis (Lond.), *12:* 1193–1196, 1991.

56. Deschner, E. E., Ruperto, J. F., Lupton, J. R., and Newmark, H. L. Dietary butyrate (tributyrin) does not enhance AOM-induced colon tumorigenesis. Cancer Lett., *52:* 79–82, 1990.

57. Sitrin, M. D., Halline, A. G., Abrahams, C., and Brasitus, T. A. Dietary calcium and vitamin D modulate 1,2-dimethylhydrazine-induced colonic carcinogenesis in the rat. Cancer Res., *51:* 5608–5613, 1991.

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