Journal of Cellular Biochemistry 25S:136–141 (1996)

# Inherited and Acquired Risk Factors in Colonic Neoplasia and Modulation by Chemopreventive Interventions

**Martin Lipkin,[1]* Kan Yang,[1] Winfried Edelmann,[2] Harold Newmark,[1] Kun-Hua Fan,[1] Mauro Risio,[3] and Raju Kucherlapati[2]**

[1]Strang Cancer Research Laboratory, Rockefeller University, New York, New York
[2]Albert Einstein College of Medicine, Bronx, New York
[3]Centro Ricerche Neoplasie Apparato Digerente "L. Novello Miglino," Ospedale S. Giovanni Vecchio, Torino, Italy

**Abstract** The progressively abnormal development of epithelial cells prior to tumor development leads to widely differing chemopreventive approaches. The diversity of these approaches has resulted in different assays to measure the activities of the agents. To apply these assays to preclinical studies, we have developed rodent models in which different stages of evolution of colonic neoplasia are expressed. In one model mice carrying a truncated Apc allele with a nonsense mutation in exon 15 have been generated by gene targeting and embryonic stem cell technology (Apc1638 mice). These mice develop multiple gastrointestinal lesions including adenomas and carcinomas, focal areas of high grade dysplasia (FAD) and polypoid hyperplasias with FADS.

The incidence of inherited colonic neoplasms has now been modulated by a chemopreventive regimen. Colonic lesions significantly increased in Apc1638 mice on a Western-style diet, compared to Apc1638 mice on AIN-76A diet which has lower fat content and higher calcium and vitamin D. These studies have also been carried out in normal mice, and have demonstrated without any chemical carcinogen that a Western-style diet induced colonic tumorigenesis. Modulation of cell proliferation has also been induced by Western-style diets in other organs including mammary gland, pancreas and prostate. These findings are leading to the development of new preclinical models for evaluating the efficacy of many classes of chemopreventive agents. J. Cell. Biochem. 25S:136–141. 

**Key words:** acquired risk; chemoprevention; colon; genetic risk; neoplasia

Principles that have guided the development of assay systems for chemoprevention studies include the following: a) Chemopreventive agents have activities during different stages in the evolution of neoplasia. b) This leads to different mechanisms through which the chemopreventive agents have activity, and to different assays of measurement, i.e., intermediate endpoints or biomarkers. c) Many chemopreventive agents have activities via several mechanisms, leading to the development and expression of multiple biomarkers.

*Correspondence to: Martin Lipkin, MD, Strang Cancer Research Laboratory, Rockefeller University, 1230 York Avenue, New York, NY 10021.

Received 11 December 1995; Accepted 3 April 1996

## CLASSES OF CHEMOPREVENTIVE AGENTS, MECHANISMS OF ACTION AND IDENTIFICATION BY INTERMEDIATE BIOMARKERS

In Table I chemopreventive agents are classified according to postulated mechanisms of action; and intermediate endpoints are enumerated that identify the actions of the chemopreventive agents [1]. In Table I, for completeness group 1 lists environmental and dietary cancer-related causes of neoplasia, together with assays for identification that are under study. In groups 2 and 3, some of the chemopreventive agents that reduce oxidative damage to DNA are summarized together with relevant biomarkers being studied. The antioxi-

**TABLE I. Intermediate Biomarkers in Chemoprevention Studies**

| Chemopreventive approaches | Postulated mechanism | Biomarkers |
|---|---|---|
| 1 Avoidance of known cancer-related agents in the environment<br>tobacco products<br>high dietary fat, calories<br>chemicals, *e.g.,* aflatoxin heterocyclic amines<br>viruses, *e.g.,* hepatitis B, HIV | Reduce DNA damage and/or promotion | Reduced urinary and blood markers that identify specific agents, *e.g.,* DNA adducts, oxidation and alkylation products |
| 2 Antioxidants<br>*e.g.,* tocopherols, ascorbate, carotenes, Se | Reduce risk of excessive oxidative DNA damage and cell toxicity | Reduced DNA oxidative metabolites in blood and urine, proliferation, acf (fad) |
| 3 Beta carotene | Reduce oxidation reactions | Reduce hyperproliferation, modulate cell differentiation |
| 4 NSAIDs, plant phenolic compounds, flavonoids conjugated linoleic acid, omega-3 (n-3) fatty acids | Inhibit arachidonic acid metabolism, oxidative reactions | Reduce lipoxygenase, cyclooxygenase activity, inflammation, proliferation |
| 5 Isothiocyanates, dithiolthiones, flavones, indoles, sulfur compounds | Alter enzyme detoxification | Modified carcinogenic metabolites in biological fluids, urine |
| indole 3 carbinol | Increase 2-OH estradiol oxidation | Increased urinary 2-OH/16-OH estradiol ratio |
| d-glucarates | Inhibit glucuronidase glucuronidase | Increase urinary glucuronide excretion, *e.g.,* estrogens |
| 6 Calcium, Vitamin D | Reduce bile acid, fatty acid irritation in colon, proliferation | Modified gene expression, reduced hyperproliferation, cytotoxicity, increased serum 25-OH Vitamin $D_3$ |
| 7 Difluromethylornithine | Inhibit ornithine decarboxylase | Reduce ODC levels associated with cell proliferation |
| 8 Dehydroepiandrosterone | Reduce G-6PDH activity | Reduce carcinogen activation, cell proliferation |
| 9 Retinoids | Induce cell differentiation | Reverse squamous metaplasia, modulate cell differentiation markers, leucoplakia |
| 10 Terpenes | Reduce oncogene isoprenylation and ubiquinone synthesis | Modulation of HMG-CoA metabolic pathways and products |
| 11 Antiestrogens (*e.g.,* tamoxifen) | Reduce estrogen-stimulated proliferation | Modulate proliferation, estrogen metabolism and gene expression |

dants include tocopherols, ascorbate, carotenes and selenium.

Group 4 lists numerous classes of chemopreventive compounds that inhibit arachidonic acid metabolic pathways, together with a variety of assays that are being studied to identify chemopreventive activities. Group 5 lists other classes of chemopreventive compounds that have altered enzyme detoxification in various studies, measured by modified metabolites in body fluids. Compounds with these properties are present in crucifer vegetables, garlic, onion and include isothiocyanates, dithiolthiones, flavones, indoles and sulfur compounds. They may alter phase 1 enzymes modifying the activation of potentially carcinogenic agents into less toxic and carcinogenic pathways or increase phase 2 enzymes and enzyme conjugation and excretion. Several examples are given: indole-3-carbinol altering and increasing 2-OH estradiol oxidation, measured by an increased ratio of urinary 2-OH/16-OH estradiol metabolites; and d-glucarates inhibiting glucuronidase activity yielding increased urinary glucuronide excretion.

The activities of calcium and vitamin D have previously reduced hyperproliferation in many rodent and human studies, cytotoxicity of co-

lonic epithelial cells, modified gene expression, and induced differentiation of a variety of epithelial cells. In the remaining categories other additional diverse mechanisms of chemopreventive activity, and different types of biomarkers are similarly enumerated.

## PRECLINICAL STUDIES TO TEST THE EFFICACY OF CHEMOPREVENTIVE AGENTS

### Gastrointestinal Neoplasms in Mice Carrying a Truncated Apc Allele

In the past, chemical carcinogens have generally been used in preclinical models to test the possible efficacy of chemopreventive agents. Recently however, several rodent models have been developed in which neoplastic lesions evolve without chemical carcinogens.

In the first of these rodent models we have studied mice have a targeted mutation in the Apc gene [2]. The adenomatous polyposis coli (APC) gene is important in the development of human gastrointestinal tumors. In our current studies mice carrying a truncated Apc allele with a nonsense mutation in exon 15 were generated by gene targeting and embryonic stem cell technology, and were designated Apc1638 mice.

In an initial study 49 gastrointestinal neoplasms consisting of adenomas and adenocarcinomas developed in 63% of mice carrying the truncated Apc allele. Adenomas and carcinomas were located in stomach, duodenum, jejunum, ileum and colon. Adenomas were tubular, tubulovillous, villous, and a majority of adenomas had severe dysplasias. Among the adenocarcinomas most invaded the muscularis mucosa, submucosa or inner layer of propria muscularis. Polypoid hyperplasias with dysplasias also were found in the colons of young mice, and adenomas, focal areas of dysplasias and polypoid hyperplasias were found in older mice. Thus, findings revealed a new rodent model based on a specific Apc gene mutation for the study of tumor development and its prevention in the gastrointestinal tract.

### Modulation of Colonic Lesions Induced in Apc1638 Mice by a Western-Style Diet

We recently induced modulation of the colonic lesions in these Apc1638 mice. In young Apc1638 mice colonic polypoid hyperplasias containing dysplasias were significantly increased by feeding the Western-style diet with reduced calcium and vitamin D and increased fat content [3]. Total polypoid hyperplasias in colons of young 18-week-old Apc1638 mice fed a Western-style diet were tenfold more frequent than in a control AIN-76A diet group. This appears to be the first animal model rapidly producing intestinal and colonic lesions without a chemical carcinogen, and rapidly responding to dietary modulation of developing colonic lesions. In older mice focal areas of dysplasia in the colon were significantly increased by the Western-style diet.

### Colonic Neoplasms Developing in Normal Mice on a Western-Style Diet Without the Use of Any Chemical Carcinogen

Our studies of the evolution of neoplasms in the colons of normal mice have shown that a Western-style diet low in calcium and vitamin D induced hyperproliferation and hyperplasia of colonic epithelial cells.

In normal C57BL/6J mice maintained on a Western-style diet findings parallel those seen in human colon in diseases that increase risk of colon cancer. In the rodent model they include the early development of increased mitosis, atypical mitosis, apoptosis of colonic epithelial cells, and the eventual development of colonic whole-crypt dysplasias—all induced in the rodent model by the Western-style diet without any chemical carcinogen [4]. The development of these findings at time periods throughout the entire life span of the rodents has now been quantified [4]. These findings now make it possible to evaluate the ability of numerous chemopreventive approaches to inhibit colonic neoplasia in the rodent model without a chemical carcinogen, and to utilize this information to further guide comparative measurements carried out on the human colon.

### Intermediate Endpoints Associated With Differentiation of Colonic Epithelial Cells Following Western-Style Diets

Previous short-term rodent studies have identified hyperproliferation in colonic epithelial cells following nutritional diets mimicking a Western-style diet. In a new study [5] two Western-style diets with high fat and phosphate, and low calcium and vitamin D content were fed to C57BL/6J mice for 12, 24 and 52 weeks. Diet A contained American Blend fat,

diet B contained corn oil, and diet C control AIN-76A. Chronic feeding of both nutritional stress diets revealed modified colonic epithelial cell differentiation up to 52 weeks of age. Comparisons were made between the Western-style diet and control groups for lectin SBA binding, cytokeratins AE1 and RPN 1160, and acidic mucins including sialo- and sulpho-mucins. In the colonic epithelial cells, lectin SBA binding became significantly increased in the Western-style diet groups compared to controls at all time periods. Significant increases also were found in the expression of cytokeratins AE1 and RPN 1160, and in total acidic mucins at all time periods. These results defined both structural and functional alterations that developed in differentiating colonic epithelial cells under these dietary conditions.

## STUDIES OF OTHER ORGANS IN ADDITION TO COLON

### Effect of Western-Style Diet on Early Abnormalities Associated With Breast Cancer in Mammary Gland of Mice

In addition to studies of the colon noted above, we also have begun to study the effects of Western-style diets on other organs including mammary gland structure and function. In a new study [6], mammary glands of female C57BL/6J mice were analyzed after feeding a Western-style diet or control AIN-76A diet for periods up to 20 weeks and carrying out morphometric and microautoradiographic measurements. By 14 weeks and 20 weeks of feeding, the number of terminal ducts in the mammary glands of the Western diet mice significantly increased compared to the control group. This is the area where carcinomas characteristically develop in rodent models and in humans. Moreover, there was a significant increase in the [$^3$H]dThd labeling index of mammary terminal ductal epithelial cells after 14 and 20 weeks of Western-style diet administration. Thus, the Western-style diet induced both increased epithelial cell proliferation and increased numbers of terminal ducts in female mice when fed during young adult growth and development. The findings raise the possibility that in humans the ingestion of a diet containing low calcium and vitamin D content might induce similar changes during the early development of human mammary glands, which is of particular importance in young adolescent women. Studies related to the above can also be carried out to test the efficacy of chemopreventive agents on mammary gland development in human subjects.

### Hyperproliferation in Exocrine Pancreas and Prostate of Mice Induced by a Western-Style Diet

We have also begun to study the effects of a Western-style diet with increased fat and low calcium and Vitamin D on epithelial cell proliferation in pancreas, prostate and bladder of C57BL/6J mice [7]. After feeding a Western-style diet for periods up to 16 weeks mice were infused with BrdU for 72 h using subcutaneous Alzet pumps. Findings revealed: In pancreas unchanged number of pancreatic ducts and acini in mice on Western-style diet or AIN-76A control diets; however BrdU-labeling indices of epithelial cells lining pancreatic inter- and intralobular ducts, and centroacinar cells significantly increased in Western-style diet compared to control diet groups at all time periods. In prostate BrdU-labeling indices significantly increased in anterior and dorsal but not in ventral lobes in Western- style diet compared to control diet groups, after feeding Western-style diet for 16 weeks. In bladder epithelial cell BrdU-labeling indices were unchanged in Western-style diet and control groups. Findings are thus similar to Western-style diet effects on colon and mammary gland, further suggesting a role of Western diets in human pancreatic and prostatic carcinogenesis, and chemopreventive strategies that can be considered.

### The Development of Clinical Trials Measuring Biomarker Modulation and Adenoma Recurrence, and Problems to be Considered in These Trials

Because of the success of preclinical studies in demonstrating efficacy of numerous chemopreventive agents in various organs, further clinical trials are currently being planned and are underway to evaluate possible chemopreventive efficacy of the agents in human subjects. In the colon investigators are attempting to standardize biomarkers to facilitate their use in large studies [8]; and they are also beginning to measure the effects of chemopreventive regimens on the recurrence of colonic adenomas. The latter clinical trials will attempt to measure whether the regrowth of adenomas is affected by the chemopreventive agent or agents being tested. Clinical trials of this type have

great potential for evaluating the efficacy of chemopreventive agents, but various limitations are now known to be present in carrying out clinical adenoma trials and in interpreting their results [1].

A major problem in designing a clinical adenoma trial is whether it is capable of actually measuring the activity and effect of the chemopreventive agent being tested. Colonic adenomas develop in the colons of humans over a duration of 20–30 years, evolving and progressing through multiple stages of abnormal cell development: from normal cells, to cells that progressively accumulate multiple genetic and metabolic abnormalities as they progress through initiation, promotion, and progression stages to tumors. Current clinical trials are now only able to measure the regrowth of small adenomatous tumors which arise from previously transformed adenomatous cells; and currently those measurements can only be carried out during a short three- or four-year period, through a small window of observation of short duration that measures the late stage of regrowth of transformed cells (Fig. 1). The clinical trial will not measure whether the chemopreventive agent inhibited genotoxicity that occurred years earlier, nor cellular metabolic abnormalities involved in tumor promotion over a long duration, nor in the progression of adenoma cells to carcinomas.

Among the numerous classes of chemopreventive substances, naturally occurring compounds generally have weaker activities compared to pharmaceutical agents, but are generally safer to administer to large populations. Many naturally occurring substances characteristically have their activity in cells that are normal or near-normal. Therefore chemoprevention studies that use a 3–4-year window of observation of adenoma cell regrowth, measuring transformed cells accumulating above the mucosal surface, are likely to require potent chemopreventive agents with potentially higher levels of toxicity targeted at mechanisms affecting cells in later stages of abnormal development, in order to achieve a rapid inhibitory effect on regrowth of the transformed adenoma cells.

To design clinical trials that can accurately test the utility of diverse classes of chemopreventive agents, it may be advisable in the future to carry out studies a) of longer duration; b) beginning at earlier ages; c) that test the mechanisms and specific stage of abnormal cell development that preclinical studies have shown are modulated by the agent being tested (Fig. 2). By addressing these questions and by designing clinical trials that are appropriate for testing the known preclinical activities of specific agents, these agents can be accurately evaluated in human populations.

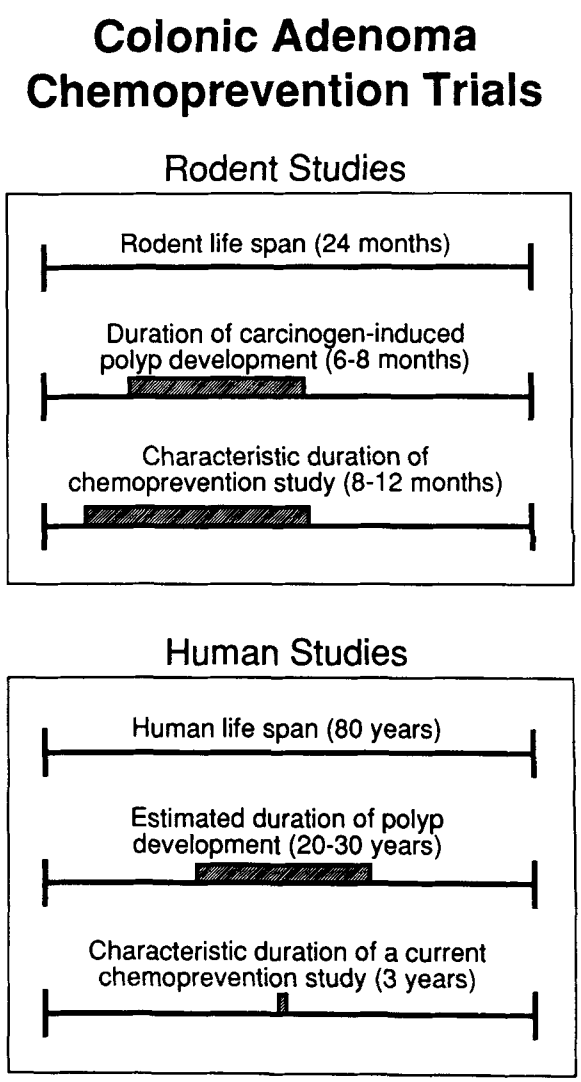


**Fig. 1.** Diagram of rodent life span, the typical duration during which carcinogen-induced colonic tumors develop, and during which chemoprevention studies are carried out. Previous chemoprevention studies in rodents have characteristically administered the agent to be tested over a large part of the rodent's life span, beginning at any early age. In humans, adenomas develop over a long duration, evolving through multiple stages of abnormal cell development: from normal cells to cells that progressively accumulate multiple genetic and metabolic defects involved in genotoxicity, and in the initiation, promotion and progression of the cells to tumors. However, current clinical adenoma trials are only able to measure the regrowth of small adenomatous tumors during a late stage of abnormal cell development as the transformed cells accumulate above the surface of the colonic mucosa [1].

# Future Chemoprevention Trials

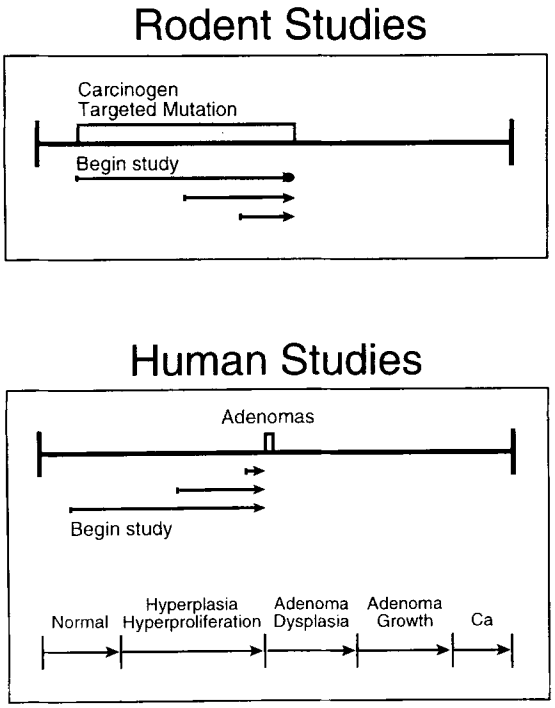


**Fig. 2.** In Figure 2 suggestions for future rodent and human studies are diagrammatically represented. Preclinical rodent studies have classically identified efficacy of chemopreventive agents after the agents were given during a large part of the animals' life span, beginning at a young age. This demonstrated tumor-inhibitory activity that could occur during early or late stages of evolution of the tumors. Thus, agents that were able to inhibit early genotoxic events could show efficacy in the same rodent model as agents that inhibited late stages of cell transformation. In future rodent studies the chemopreventive agents can be tested to determine if they inhibit early genotoxicity, later metabolic events or the progression of adenomas to carcinomas, thus guiding the development of human clinical trials by evaluating when the agents should be administered to human subjects in the clinical trials. Human clinical adenoma trials now are only able to measure efficacy of chemopreventive agents against the late-stage accumulation of transformed adenoma cells above the surface of the colonic mucosa, during a relatively short period of observation. To test natural substances and the many different classes of chemopreventive agents now available which act through widely differing mechanisms, it may be necessary to begin clinical trials at earlier ages for longer durations. It may be further advisable to test population groups separately, when they are shown to have the same pre-adenoma abnormalities measuring transition to the next stage; and separately to test the progression of adenomas to carcinomas [8].

## REFERENCES

1. Lipkin M: "Strategies for colon cancer prevention." (1995) New York: In: Cancer Prevention, Eds. Bradlow LH, Osborne M, Veronesi U. Annals of the New York Academy of Sciences 768:170–179.
2. Fodde R, Edelmann W, Yang K, van Leeuwen C, Carlson C, Renault B, Breukel C, Alt E, Lipkin M, Khan P, Kucherlapati R (1994): A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors. Proc Natl Acad Sci U S A 91:8969–8973.
3. Lipkin M, Yang K, Fan K, Newmark H, Edelmann W, Fodde R, Leung D, Kucherlapati R (1995): Modulation of colonic lesions induced in Apc1638 mice by a Western-style diet. Proc Am Assoc Cancer Res 36:596.
4. Risio M, Lipkin M, Newmark H, Yang K, Rossim F, Steele VE, Boone CW, Kelloff GJ (1996): Apoptosis, cell replication, and Western-style diet-induced tumoregenesis in mouse colon. Cancer Research 56:4910–4916.
5. Yang K, Fan K, Newmark H, Leung D, Lipkin M, Steele VE, Kelloff GJ (1996): Cytokeratin, lectin and acidic mucin modulation in differentiating cobonic epithelia P cells of mice after feeding Western-style diets. Cancer Research 56:4644–4648.
6. Xue L, Newmark H, Yang K, Lipkin M (1996): Model of Mammary gland hyperproliferation and hyperplasia induced by a Western-style diet. Nutr Cancer 26:281–287.
7. Xuel, Yang K, Newmark KH, Leung D, Lipin M. Journal of National Cancer Inst. in press.
8. Boone C, Kelloff, G (1994): Quantitative pathology in chemoprevention trials: Standardization and quality control of surrogate endpoint biomarker assays for colon, breast, and prostate. J Cell Biochem 19 (Suppl): 10–22.
9. Lipkin, M: Strategies for intervention with chemopreventive agents. (1996) Int J Cancer, 69:64–67 (Pred. Oncol).