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Newer Approaches to the Prevention of Gastrointestinal Cancer

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To identify the efficacy of chemopreventive agents and their effect on progressive stages of colonic preneoplasia and tumor evolution, new preclinical models have recently been developed. Some of these models have targeted mutations that modify the incidence and progression of neoplastic lesions. In one model of inherited predisposition to colon cancer mice carrying a truncated *Apc* allele with a nonsense mutation in exon 15 (*Apc1638* 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 intestinal neoplasms including colonic significantly increased in *Apc1638* mice on a Western-style diet with higher fat content and lower calcium and vitamin D compared to AIN-76A diet. *Min* mice with an *Apc* mutation had a reduced incidence of intestinal tumors after Sulindac administration. Mice carrying a targeted mutation in the gene *Mcc* (mutated in colorectal cancer) develop multiple types of neoplasms including adenocarcinomas, focal areas of gastrointestinal dysplasia, papillomas of the forestomach and tumors in other organs including lung, liver and lymphoid tissue. Feeding a Western-style diet to *Mcc* mutant mice also significantly increased gastrointestinal lesions. In normal mice a Western-style diet also induced whole-crypt colonic dysplasias without any chemical carcinogen. Western-stlye diets have now induced epithelial cell hypoproliferation in other organs including mammary gland, pancreas and prostate. These findings are leading to the development of new preclinical rodent models to aid the analysis of genetic and environmental factors leading to neoplasia, and are assisting clinical studies to evaluate the chemopreventive efficacy of specific nutrients and pharmacological agents. Human studies of chemopreventive regimens are underway to evaluate efficacy in colon, breast, esophagus, stomach, cervix, liver, lung and other organs, and these clinical trials are underway in many countries worldwide.

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GASTROINTESTINAL NEOPLASMS IN MICE INDUCED BY A TARGETED *APC1638* MUTATION

Preclinical models have previously used chemical carcinogens to test the possible efficacy of chemopreventive agent. Recently, however, new rodent models have shown neoplastic lesions evolving without chemical carcinogens. Our published studies utilizing these models are summarized below. In the first of this series mice had a targeted mutation in the *Apc* cene. The adenomatous polyposis coli (*Apc*) gene is important in the development of human gastrointestinal tumors. Mice carrying a truncated *Apc* allele with a nonsense mutation is exon 15 were generated by gene targeting and embryonic stem cell technology, and were designated *Apc1638* mice.[1)]

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, mostly in small intestine. Adenomas were tubular, tubulovillous, villous and a majority had severe dysplasias (Table 1). The adenocarcinomas mostly invaded the muscularis mucosa, submucosa or inner layer of propria muscularis. Polypoid hyperplasias with dysplasias also were found in the colons of young mice; adenomas, focal areas of dysplasias and polypoid hyperplasias were found in older mice. These findings revealed a new rodent model based on a specific *Apc* gene mutation for the study of tumor development in the gastrointestinal tract and its prevention.

COLONIC LESIONS IN APC1638 MICE INCREASED BY A WESTERN-STYLE DIET

We recently modified the development of the colonic lesions in these *Apc1638* mice with diet. Young *Apc1638* mice developed colonic polypoid hyperplasias contatining dysplasias; older mice developed carcinomas throughout the gastrointestinal tract. Both were significantly increased by feeding a Western-style diet characterized by reduced calcium and vitamin D and increased fat content, and were decreased by decreasing fat content and increasing dietary calcium and vitamin D. This was the first animal model rapidly producing intestinal and colonic lesions without a chemical carcinogen, which rapidly responded to dietary modulation of developing colonic lesions.[2)]

INTESTINAL LESIONS IN MIN MICE DECREASED AFTER SULINDAC

For chemopreventive efficacy Sulindac also has been studied in *Min* mice, a strain with a mutated *Apc* gene that develops intestinal adenomas at a young age. In *Min* mice tumors develop more rapidly and the mice have a shorter life span compared to *Apc1638* mice; thus the two models should differ somewhat in their potential for screening chemopreventive agents. This agent significantly reduced the number of tumors per mouse after feeding Sulindac in drinking water. Mucosal PGE_2 and Cox-2 protein levels appeared to be higher in the small intestine of the *Min* mice compared to normal control mice, and treatment with Sulindac reduced PGE_2 and Cox-2 protein and increased apoptosis.[3)]

NEOPLASMS IN MICE CARRYING A MUTATION IN THE Mcc GENE: GASTROINTESTINAL LESIONS INCREASED BY A WESTERN-STYLE DIET

The gene *Mcc* is located on human chromosome 5q21, and is frequently mutated in colorectal tu-

mors. To determine the role of *Mcc* in normal development and in the onset and progression of colorectal cancer, the mouse gene was isolated and a gene targeting construct made[4] by inserting of a neomycin phosphotransferase expression cassette into the 11th exon of the gene. Transfection of mouse embryonic stem cells with the construct yielded one clone in which the *Mcc* gene was genetically modified. Mice which are heterozygous for the modification were obtained and interbred, and mice homozygous for the *Mcc* gene modification were also obtained. These mice had no detectable *Mcc* protein, suggesting that the gene is not required for normal development. *Mice* carrying the Mcc mutation developed neoplasms, including adenocarcinomas, focal areas of gastrointestinal dysplasia (fad), papillomas of the forestomach, and tumors in other organs, including lung, liver, and lymphoid tissue, which developed between 12~22 months of age. These results suggested that *Mcc* has a critical role in the growth regulation of a number of cell types. Feeding a Western-style diet to the *Mcc* mutant mice also resulted in a significant increase in gastrointestinal lesions, as occurred in *Apc1638* mice. The proliferative index and size of proliferative compartment significantly increase in flat intestinal mucosa of mice carrying the *Mcc* mutation. The results thus suggested that the *Mcc* gene product has an important role in the onset and progression of colonic and extracolonic tumors.[4]

NORMAL MICE: COLONIC WHOLE CRYPT DYSTPLASIAS INDUCED BY A WESTERN-STYLE DIET

Previous studies of the development of neoplasms in the colons of normal mice showed that Western- style diets with the same nutrient modifications noted above induced hyperproliferation and hyperplasia of colonic epithelial cells. Two Western-style diets with high fat content and low calcium and vitamin D were fed to normal C57BL/6J mice; the Western-style diets contained either American Blend fat or corn oil. Hyperproliferation and hyperplasia were followed by other changes in the colon, including dysplasias, similar to those seen in the human colon in diseases that increase risk of colon cancer; they occurred without administering any chemical carcinogen.

The Western-style diets also induced the development of, atypical mitosis, and the eventual development of colonic whole-crypt dysplasias in normal rodent colon. The development of these findings throughout the entire life span of the rodents has now been quantified,[5] making it possible to analyze molecular changes occurring during the various stages of evolution of neoplasia; to study the ability of various chemopreventive strategies to inhibit colonic neoplasia in new rodent models without chemical carcinogens; and to utilize this information to aid the interpretation of findings observed in the human colon.

NORMAL MICE: DIFFERENTIATION -ASSOCIATED STRUCTURAL AND FUNCTIONAL PROPERTIES OF COLONIC EPITHELIAL CELLS MODIFIED BY WESTERN-STYLE DIETS

Previous short-term studies of normal rodent colon identified hyperproliferation in colonic epithelial cells following Western-style diets. In a recent study,[6] chronic feeding of both of the above Western-style diets revealed modified colonic epithelial cell differentiation in the colon for a duration of 52 weeks, or half of the animals' life span. Comparisons were made for lectin SBA binding, cytokeratins AE1 and RPN 1160, and acidic mucins including sialo- and sulpho -mucins. In colonic epithelial cells, lectin SBA binding significantly increased in the Westen-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 adverse dietary conditions, eventually leading to dysplastic lesions[6] in the colons of the same mice.

NORMAL MICE: CELL PROLIFERATION AND HYPERPLASIA IN MAMMARY GLAND INCREASED BY A WESTERN-STYLE DIET

In new studies,[7,8] mammary glands of female C57BL/6J mice were analyzed after feeding a Western-style diet or control AIN-76A diet for short- durations up to 20 weeks, with mammary glands removed for morphometric and microautoradiographic measurements. The number of terminal ducts in the mammary glands of mice on the Western-style diet significantly increased compared to the control group. This is the region in mammary gladn where precancerous lesions and carcinomas characteristically develop in rodent models and in humans. Moreover, there was a significant increase in [^{3}H]dThd labeling indices of mammary terminal ductal epithelial cells in mice fed the Western-style diet. Thus, the Western-style diet induced bothe 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 the ingestion of a diet low in calcium and vitamin D might induce similar changes during the early development of mammary gland in adolescent young women where calcium and vitamin D intakes are characteristically low and cell proliferation is increased, which could facilitate the later evolution of neoplastic lesions.

NORMAL MICE: CELL PROLIFERATION IN EXOCRINE PANCREAS AND PROSTATE OF MICE INCREASED 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.[9~11] After feeding a Western-style diet for short durations up to 16, weeks mice were infused with BrdU for 72 hours using subcutaneous Alzet pumps. In pancreas we found an 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. These corresponded to regions in the pancreas where carcinomas develop in rodent models and in humans. In prostate BrdU-labeling indices significantly increased in anterior and dorsal but not ventral lobes in Western-style diet, compared to control diet groups, after feeding Western- style diet for 16 weeks. This also corresponded to the regions in prostate gland where carcinomas develop in humans and rodent models. In bladder, epithelial cell BrdU-labeling indices were not significantly modified in Western-style diet and control groups. Western-style diet effects are thus similar in colon, mammary gland and pancreas, further suggesting a role of Western diets in human carcinogenesis in these organs and additional strategies that can be considered for the chemoprevention of cancer.

NEW CLINICAL TRIALS THAT EVALUATE THE EFFICACY AND SAFETY OF CHEMOPREVENTIVE REGIMENS IN HUMAN SUBJECTS

In addition to the preclinical studies noted above,

many others are underway evaluating the efficacy and safety of a wide variety of potential chemopreventive regimens. The preclinical studies of many of the regimens have now advanced to clinical trials carried out in human subjects. These clinical trials include the administration of *whole diets, individual nutrients, or pharmaceutical compounds.* The *dietary regimens* that attempt to inhibit tumor development have involved mainly lowering dietary fat content, and increasing intakes of fiber, fruits and vegetables [eg, summarized in 12]. The *individual nutrients or combinations* of the nutrients under study, and examples of the many categories of *pharmaceutical agents* now being evaluated are given in Table 2. The numerous mechanisms through which these agents have chemopreventive activities are summarized in current reviews [e.g., 12~18]. These studies are now underway in many countries and geographic regions as illustrated in Table 3.

Most of the studies carried out in human subjects have been aided by studying the short-duration modulation of intermediate endpoint biomarkers rather than by studying the evolution of tumors over a long duration. When assessing the utility of biomarkers in human clinical trials, the closer the biomarker is to the development of cancer the more useful it will be. Thus, biomarkers describing the very advanced stages of precancerous disease (e.g., dysplasias) are most closely associated with the evolution of cancer; the modulation of early-stage endpoints (e.g., cell proliferation or arachidonic-acid metabolic pathways) can also give useful information, by identifying mechanisms of activity through which the chemopreventive agents act in humans, and whether the activity is the same as that found in preclinical models where tumors are inhibited. Intermediate endpoints now under study in various clinical trials inclue: late-stage precancerous dysplasias (in studies of breast, cervix, oesophagus, lung, oropharynx, skin, and stomach); recurrence of precancerous lesions (in cervix, colon, and oral mucosa); micronuclei (in oesophagus, lung, and oral mucosa); cell proliferation (in oesophagus and colon); and cancer development (in breast, colon, oesophagus, lung, liver, and skin).[12~18)]

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Table 1. Histologic categories of tumors in the gastrointestinal tract of *Apc1638* mice[19)]

Histologic Category	Number of Growths(%)
Adenoma	24(100%)
Tubular	14(58%)
Tubulovillous	6(25%)
Villous	4(17%)
Adenocarcinoma	25(25%)
Carcinoma in situ	7(28%)
Early invasive carcinoma	11(44%)
Advanced invasive carcinoma	7(28%)
Total	49

Table 3. Countries with chemoprevention intervention trials[12,15,16]

Country	Organ site studied
USA	Breast, cervix, colon, prostate head and neck, lung, oropharynx, skin
Europe	Breast, colon, cervix, head and neck, lung, stomach
Australia	Colon, lung
Africa	Skin, liver
Canada	Breast, colon, oropharynx
China	esophagus, lung, liver, oropharynx
Korea	Stomach
India	Oral
South America	Stomach
USSR	Oropharynx, esophagus, lung

Table 2. Chemoprevention trials underway and planned[12,15,16]

Clinical Chemoprevention Trial	Organ Site(s) Studied
Nutritional supplements And modified diets	
Calcium	Breast, colon, esophagus
β-carotene and other carotenoids	Breast, cervix, colon, esophagus, lung, oral, skin, stomach
Dietary modifications	Breast, colon, skin
Indole-3carbinol	Cervix, larynx
Selenium	Liver, lung, skin
Vitamin A	Lung, oral, skin
Vitamin C	Colon, stomach
Vitamin D_3 (and analogues)	Colon, breast
Vitamin E	Breast, colon, oesophagus, oral, prostate
Vitamins, combined	Cervix, colon, oesophagus, lung, oral, stomach
Vitamins and Minerals combined	Colon, esophagus, lung, oral
Pharmaceutical agents	
Aspirin	Breast, colon, lung
Antibiotics	Stomach
Dehydroepiandrosterone (DHEA) Analogue 8354	Breast, prostate
2-difluoromethylonithine (DMFO)	Bladder, cervix, colon, oral, prostate
Finasteride	Prostate
18β-glycyrrhetinic acid	Liver
N acetyl 1 cysteine(NAC)	Bladder, lung
N-(4-hydroxyphenyl) retinamide(4-HPR)	Bladder, breast, cervix, lung, oral, prostate, skin
NSAIDS(ibuprofen, piroxicam, sulindac, COX-2 inhibitors)	Bladder, colon
Oltipraz	Bladder, breast, liver, lung, prostate
Retinoids, synthetic	Breast, cervix, head and neck, lung, skin
Sunscreens	Skin
Tamoxifen	Breast
Vaccinations	Liver