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New Rodent Models for Studies of Chemopreventive Agents

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Abstract Some recent studies of the effects of chemopreventive agents have begun to use new rodent models to improve the analysis of stages of colonic preneoplasia, and how chemopreventive agents modify progressive abnormal cell development. In one of the models of inherited predisposition to colon cancer, 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, which has higher fat content and lower calcium and vitamin D compared to the same mice on AIN-76A diet. In another rodent model, Min mice were treated with sulindac, which markedly reduced the incidence of intestinal tumors. A third new rodent model containing a targeted mutation in the gene Mcc (mutated in colorectal cancer) recently became available for chemoprevention studies. These mice 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 the Mcc mutant mice also resulted in significantly increased gastrointestinal lesions. These nutrient modifications also have been given to normal mice, demonstrating without any chemical carcinogen that a Western-style diet induced colonic tumorigenesis. Western-style diets also have now induced modulation of cell proliferation in other organs including mammary gland, pancreas, and prostate. These findings help develop new preclinical rodent models to aid the analysis of genetic and environmental factors leading to neoplasia, as well as new methods for evaluating the chemopreventive efficacy of specific nutrients and pharmacological agents. J. Cell. Biochem. Suppls. 28/29:144–147.

Key words: mice; gastrointestinal neoplasms; colonic lesions; Western-style diet; chemopreventive agents

GASTROINTESTINAL NEOPLASMS IN MICE INDUCED BY A TARGETED APC1638 MUTATION

In past studies, preclinical models used chemical carcinogens to test the possible efficacy of chemopreventive agents. Recently, however, new rodent models have shown neoplastic lesions evolving without chemical carcinogens.

In the first of these rodent models we studied, mice had a targeted mutation in the Apc gene [1]. The adenomatous polyposis coli (APC) gene is important in the development of human gastrointestinal tumors. These 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 [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, and villous; a majority had severe dysplasias. 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. Thus, findings revealed a new rodent model based on a specific Apc gene muta-

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tion for studying tumor development and its prevention in the gastrointestinal tract [2].

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

We recently induced modulation of the colonic lesions in these Apc1638 mice. Young Apc1638 mice developed colonic polypoid hyperplasias containing 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

Sulindac as a chemopreventive agent 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 Apc 1638 mice; thus the two models differ somewhat in their potential for screening chemopreventive agents. Min mice were fed AIN-76 diet and sulindac in drinking water, and control Min mice and mutant Min littermates received AIN-76 without sulindac. This agent significantly reduced the number of tumors per mouse. Small intestine was similar in crypt-villus morphology, proliferation, and apoptosis (TUNEL and bcl-2 expression) in Min and control mice. However, mucosal PGE_2 and cox-2 protein levels were increased in the Min mice compared to normal control mice. Treatment with sulindac reduced PGE_2 and cox-2 protein and increased apoptosis in Min mice. Sulindac was thus an effective chemopreventive agent in the Min mouse, and induced apoptosis as a possible mechanism of its antineoplastic activity [3].

NEOPLASMS IN MICE WITH A MUTATION IN THE MCC GENE: GASTROINTESTINAL DYSPLASIAS INCREASED BY A WESTERN-STYLE DIET

The gene MCC is located on human chromosome 5q21, and is frequently mutated in colorectal tumors. To determine the role of MCC in normal development and in the onset and progression of colorectal cancer, we isolated the mouse gene and made a gene targeting construct [4] by inserting 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 that 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 resulted in a significant increase in gastrointestinal dysplasias, as occurred in Apc1638 mice. The proliferative index and size of proliferative compartment significantly increased 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.

NORMAL MICE: COLONIC WHOLE CRYPT DYSPLASIAS 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 nutrient modifications noted above induced hyperproliferation and hyperplasia of colonic epithelial cells. These findings 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.

Western-style diets induced the early development of increased mitosis, atypical mitosis, increased apoptosis of colonic epithelial cells, 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 interpreting 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], 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 or corn oil. Chronic feeding of both 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 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 adverse dietary conditions, eventually leading to dysplastic lesions [5] 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 gland 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 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 the ingestion of a diet low in calcium and vitamin D might induce similar changes during the early development of mammary glands in adolescent young women, which 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 chemopreventive strategies that can be considered.

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