ACCELERATED SHORT COMMUNICATION

Suppression of intestinal polyp development by low-fat and high-fiber diet in Apc\(^{\Delta 716}\) knockout mice

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Most epidemiological and animal studies show a positive correlation of the dietary intake of fat with the incidence of colon cancer, whereas an inverse correlation of the dietary intake of fiber. In rats fed a diet low in fat and high in wheat bran fiber and calcium, a significant decrease was reported in the number of azoxymethane-induced aberrant crypt foci compared with those fed a high-fat, low-fiber and low-calcium diet. Mutations in the human APC gene play a key role, not only in familial adenomatous polyposis, but also in many sporadic cancers of the entire digestive tract. We previously constructed a mouse strain Apc\(^{\Delta 716}\), carrying a truncation mutation at codon 716 of the Apc gene, the homolog of human APC (10). The heterozygous mice developed numerous intestinal polyps, and all microadenomas dissected from the earliest polyps had already lost the wild-type allele, indicating the loss of heterozygosity. Using these Apc\(^{\Delta 716}\) knockout mice, we have investigated the effect of a low-fat and high-fiber diet (LRD for ‘low-risk’ diet) on intestinal polyposis, and compared it with that of a high-fat and low-fiber diet (HRD for ‘high-risk’ diet). The mice were fed either diet for 7 weeks, and the number and size of intestinal polyps were scored. The LRD-fed mice had fewer polyps than the HRD-fed mice, by 36% in the small intestine and by 64% in the colon. As for the polyp size distribution, there was no significant difference between the HRD- and LRD-fed mice. These results indicate that LRD can suppress intestinal polyposis compared with HRD which does not, and suggest that its suppression is at the initiation of polyp formation. This is likely to be due to a decreased frequency of loss of heterozygosity, rather than a retarded growth of the polyp adenomas.

Colon cancer is one of the leading causes of cancer mortality in the US (1) and other developed countries. Yet its incidence varies around the world by as much as twenty fold, suggesting that environmental factors, and especially diet plays a very significant role in its etiology. Epidemiological studies have shown that diets high in fat and low in fiber are associated with the higher incidence of colon cancer in Western populations (2–5). Previous studies demonstrated that the number of carcinogen-induced preneoplastic colonic lesions were significantly lower in rats fed a low-fat, high-fiber and high-calcium diet than in those fed a high-fat, low-fiber and low-calcium diet (6). It appears that the etiology of sporadic colon cancer cases in humans is predominantly environmental rather than genetic, whereas the pathogenesis of colonic neoplasms in hereditary diseases such as familial adenomatous polyposis (FAP*) or hereditary nonpolyposis colorectal cancer (HNPCC) involves germline mutations in the APC or various DNA repair genes, followed by additional somatic mutations in the remaining wild-type allele (7–9). Mutations in the APC gene are found not only in FAP patients, but also in sporadic colon cancer and HNPCC (7–9).

To evaluate the preventive effect of a low-fat and high-fiber diet on intestinal tumorigenesis in a genetically well-defined animal model system, we used the Apc\(^{\Delta 716}\) knockout mice we had constructed earlier (10). Although the homozygous mutants die in utero within 8 days of gestation, heterozygotes survive beyond sexual maturation and develop numerous intestinal polyps. The earliest polyps arise multifocally during the third week after birth, and new polyps continue to appear thereafter. The nascent polyps show a characteristic morphology; a single layer of microadenoma cells attached inside the normal villus epithelium. All microadenomas at the earliest stage already show the loss of the full-length wild-type Apc allele; i.e., the loss of heterozygosity (LOH). These results are consistent with the negative data against the dominant-negative mechanism of polyp formation (11). Accordingly, this mutant mouse strain is an ideal experimental model for human FAP, whose polyps develop into malignant cancer eventually, unless surgically removed. Using this model, we investigated previously the effects of the meat-derived carcinogens, heterocyclic amines (12), and a chemopreventive fish oil ingredient, docosahexaenoic acid (DHA [13]). Recently, we described morphological and molecular processes of polyp formation in these knockout mice (14).

The high-risk diet (HRD) and low-risk diet (LRD) used in this study are identical to those used previously (6) except that the calcium content was the same in both diets (200 g/kg of fat mixture, but only 25 g/kg of wheat bran. In contrast, LRD contained only 50 g/kg of fat mixture, but 200 g/kg of wheat bran. Contents of all other ingredients were based on a semisynthetic diet AIN-76A, and were identical between HRD and LRD. The Apc\(^{\Delta 716}\) heterozygous mice (10; backcross generation N\(_6\) from 129/Sv to C57BL/6 background; 7 randomized mice/group; 4 males and 3 females/group) were fed ad libitum with HRD or LRD for 3 weeks of age immediately.
after weaning to 10 weeks of age (i.e., 7-week feedings). Reflecting the diet difference, the two groups of mice ate different amounts, and resulted in different body wt ranges. On average, an HRD-fed mouse ate 69.8 g and reached the mean body weight of 24.6 g (21.8 g for female and 26.7 g for male) at the time of killing, whereas an LRD-fed mouse ate 119 g and reached 27.1 g (24.6 g for female and 29.0 g for male). These weight differences suggest that mice on LRD had more calories and a relatively greater intake of wheat bran fiber, while differences in fat intake were less than the dietary analysis would indicate.

At 10 weeks of age, the mice were killed, and the number and size of the polyps were scored in a blind manner under a dissection microscope as described previously (10). As shown in Figure 1, LRD significantly decreased the polyp number of the small intestine by 36%, and that of the colon by 64% compared with HRD. In other words, HRD increased the polyp number by 56% compared with LRD in the small intestine, and by 182% in the colon. These differences in polyp numbers between LRD and HRD are statistically significant by Student’s t-test with the P values of 0.05 and 0.03 for the small intestine and colon, respectively. As shown in Figure 2, however, there was no significant difference between the two groups in terms of the polyp size distribution. These results taken together, suggest that LRD suppresses polyp multiplicity, but has little effect on their growth once the nascent polyps are formed. Not surprisingly, histological pictures of the polyps at various stages of development showed no significant difference between those developed in the HRD- and the LRD-fed mice (data not shown).

Various diets and chemical compounds have been tested for their activities in colon tumorigenesis. For example, a Western-style diet containing high-fat and phosphate, and low calcium and vitamin D causes hyperproliferation and hyperplasia in the mouse sigmoid colon, and these are reversed by the addition of calcium sources (15). However, a typical high-risk Western diet is not low in calcium, although the demand for calcium is very high because sizeable amounts of calcium are required to detoxify the high levels of fat. This drain of calcium causes the biological effect similar to that caused by consumption of low calcium diet. Accordingly, we did not reduce the calcium content in HRD in our experiments. In the Min mice (16), soyabean-derived Bowman–Birk inhibitor (BBI) suppresses tumor multiplicity by 40 to 50% (17). It is interesting that BBI appears to reverse the initiating event in tumorigenesis although its precise mechanism remains to be investigated (18). Using the Apc\textsuperscript{\textDelta 716} knockout mice, we
demonstrated earlier that 3% DHA in the diet suppresses the polyp number and size significantly, but only in the females. The results suggest that a metabolite of DHA suppresses the growth of polyp adenomas, rather than the initiation of polyp formation (13).

On the other hand, nonsteroidal anti-inflammatory drugs (NSAIDs) sulindac and piroxicam have been shown to reduce the number of polyps in the Min mice (19,20). Recently, we demonstrated that cyclooxygenase II (COX-2) is induced in early stage polyps in the Apc<sup> mutant knockout mice, and that introduction of a COX-2 gene knockout mutation dramatically reduces both the polyp number and size, and proved that COX-2 plays a key role in polyp development (21). Moreover, a novel selective inhibitor of COX-2 can mimic such gene knockout effects in a dose-dependent manner much more effectively than sulindac (21). These results opened a new possibility in the chemotherapy of human polysisis and the chemoprevention of polyp-derived cancers of the digestive system. Although a secreted form of phospholipase A<sub>2</sub> has been proposed as a candidate for a major modifier of Min, Mom-1 (22), its clinical relevance remains to be investigated.

The effect of LRD we report here is different from those of NSAIDs and COX-2 inhibitors because LRD did not affect the polyp size but decreased only their multiplicity. Although the overall effects of diets on intestinal tumorigenesis can be much more complex than a single chemical compound such as NSAIDs or COX-2 inhibitors, it is reasonable to conclude that major effect of LRD on Apc<sup> mutant polyps is through a different mechanism from growth suppression of the polyp adenomas. It is rather more likely that LRD reduces the frequency of the initial event, i.e., LOH of the Apc gene as suggested by genotyping analyses of the nascent polyps in the Apc<sup> mutant knockou mice (10,12). It is conceivable that LRD affects the intestinal flora that influences intestinal mutagens and frequency of LOH, accordingly. Such a mechanism is consistent with the effects of LRD in the azoxymethane/rat colon abuant crypt foci model, in which mutations in the K-ras oncogene appear to be the triggering event (23). These results also support the US public health policy that the consumption of a low-fat and high-fiber diet would be beneficial in reducing the cancer incidence both in the general and high-risk populations such as those with FAP. While COX-2 inhibitors appear to be a novel class of promising chemopreventive agents for high-risk patients, dietary measures can strengthen chemoprevention because their mechanisms of action are different and their effects are likely to be synergistic.

Acknowledgements

We thank Zhaocheng Tang for the diet design and preparation, Rinko Toyoda for excellent technical assistance, and Hiroharu Arakawa for statistical analysis. This work was supported in part by grants from Ministry of Health and Welfare, and Ministry of Education, Science, and Culture of Japan.

References


Received on March 17, 1997; revised on June 3, 1997; accepted on June 19, 1997.