Cancer Chemother Pharmacol (2004) 54 (Suppl 1): S40–S43
DOI 10.1007/s00280-004-0885-z

**Yoshiro Niitsu · Tetsuji Takayama · Koji Miyanishi
Atsushi Nobuoka · Tsuyoshi Hayashi
Takehiro Kukitsu · Kunihiro Takanashi
Hirotoshi Ishiwatari · Tomoyuki Abe
Takahiro Kogawa · Minoru Takahashi
Takuya Matsunaga · Junji Kato**

Chemoprevention of colorectal cancer

Published online: 7 August 2004

Abstract Colorectal cancer is a disease with a high mortality rate and it has been increasing in prevalence worldwide. Chemoprevention, as well as primary and secondary prevention, for colorectal cancer have attracted much attention. Many chemopreventive trials have been performed, and several agents, including nonsteroidal antiinflammatory drugs, such as aspirin and sulindac, cyclooxygenase-2 selective inhibitors, such as celecoxib, vitamin D, folate, and calcium, have been shown to have some effect. In these chemopreventive trials, the targeted lesions used for evaluation were mainly polyps. However, the chemopreventive effects of some agents on polyps may require several years to evaluate. Further, larger polyps may not be susceptible to chemopreventive agents. Aberrant crypt foci (ACF) are tiny lesions at the earliest stage of colorectal carcinogenesis, which consist of large, thick crypts identified by dense, methylene blue staining. We succeeded in identifying human ACF in situ using magnifying endoscopy and found that the number of ACF, particularly dysplastic ACF, increased significantly from normal subjects to adenoma patients and then to cancer patients. We also found that the number, size, and dysplastic features of ACF are significantly correlated with the number of adenomas in adenoma patients. Thus, it was surmised that ACF are precursor lesions of the adenoma-carcinoma sequence in humans and that ACF may be the most appropriate lesions as targets for chemoprevention. We have shown that the number of ACF was significantly reduced in patients treated with sulindac. We are currently proceeding with a randomized, double-blind, chemopreventive trial targeting ACF.

Keywords Colorectal cancer · Chemoprevention · Aberrant crypt foci · COX-2 · Glutathione S-transferase P1-1

This work was presented at the 19th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "State of the Arts for Digestive Organs", 14–15 November 2003, Nagoya, Japan.

Y. Niitsu (✉) · T. Takayama · K. Miyanishi · A. Nobuoka
T. Hayashi · T. Kukitsu · K. Takanashi · H. Ishiwatari
T. Abe · T. Kogawa · M. Takahashi · T. Matsunaga · J. Kato
4th Department of Internal Medicine,
Sapporo Medical University School of Medicine,
South 1 West 16, Chuo-ku, 060-8543 Sapporo, Japan
E-mail: niitsu@sapmed.ac.jp
Tel.: +81-11-6112111 ext. 3260
Fax: +81-11-6127987

Introduction

Colorectal cancer is the most common cause of cancer-related death in the world, and its prevalence and mortality have been increasing [1, 25]. The search for effective modes of prevention is currently under way. Two such modes are primary prevention, which involves lifestyle modification, and secondary prevention such as screening with fecal occult testing or endoscopy. Since polyps are well-known precursor lesions of colorectal cancer, endoscopic resection of polyps is performed when they are found on colonoscopy. Another approach to reduce mortality entails the use of oral agents that can prevent cancer from developing in the colorectum. Such pharmacological prevention is called chemoprevention, and many studies using various compounds are currently being performed. However, no clearly effective agents to prevent colon cancer have been reported.

We have been testing a new strategy for chemoprevention of colorectal cancer that targets small lesions in the colorectum, aberrant crypt foci (ACF), with the aid of magnifying endoscopy. We are also proceeding with a randomized, double-blind, chemopreventive trial targeting ACF.

Representative chemopreventive trials for colorectal cancer

Colorectal cancer develops through a multistep process characterized by histopathological precursor lesions and molecular genetic alterations [20, 32]. This sequential process of carcinogenesis provides good opportunities for prevention of colorectal cancer. Many clinical trials of chemoprevention for colorectal cancer have been documented. Representative trials and chemopreventive agents are shown in Table 1; nonsteroidal antiinflammatory drugs (NSAIDs), such as aspirin and sulindac [3, 6, 7, 10, 26], and cyclooxygenase-2 (COX-2) selective inhibitors, such as celecoxib [28], vitamin D [5, 13], folate [8], and calcium [2, 5, 13], have been reported to show chemopreventive effects in human trials. In these trials, the targeted lesions for evaluation were mainly polyps. That is, the trials were to determine whether the number, size, or incidence of polyps was reduced. However, in using polyps as targets for chemoprevention, the following problems have been raised: (1) polyps are too large to be eradicated completely by chemopreventive agents; (2) evaluation of chemopreventive effects on polyps generally requires a long period; therefore compliance tends to become low partly due to adverse effects. To overcome these problems, lesions that appear earlier than polyps in colorectal carcinogenesis should be used as targets.

Aberrant crypt foci

ACF were first described by Bird as lesions consisting of large, thick crypts in methylene blue-stained specimens of colon from mice or rats treated with a colorectal carcinogen [4]. ACF similar to those in rodents have also been reported in surgically resected colorectal mucosa in humans [21, 22, 24]. We succeeded in identifying human ACF using magnifying endoscopy (Fig. 1), and found that the number of ACF, particularly dysplastic ACF, increased significantly from normal subjects to adenoma patients and then to cancer patients. We also found that the number, size, and dysplastic features of ACF are significantly correlated with the number of adenomas in patients with adenoma. Moreover, in some patients we found that polyps overlapped ACF. Thus, we could demonstrate that ACF are precursor lesions of the adenoma-carcinoma sequence in humans [29].

It has been reported that K-ras mutations can be detected in 57–80% of human sporadic ACF [23, 29, 33]. Smith et al. found no APC mutation in sporadic ACF, but did detect the mutation in 7% of familial adenomatous polyposis (FAP) ACF [27]. Otori et al. found no APC mutation in nondysplastic ACF, but did detect the mutation in 30% of dysplastic ACF [18]. We found that sporadic ACF are frequently positive for K-ras mutation and negative for APC mutation, and conversely that FAP ACF are positive for APC mutation and negative for K-ras mutation, applying high sensitivity methods [30]. These results support the notion that ACF are precursor lesions of colorectal cancer in both sporadic and FAP carcinogenesis, and that their mechanisms of cancer development are not necessarily the same. Since ACF are small lesions with a simple genetic alteration (K-ras mutation or APC mutation), in contrast to polyps with multiple gene abnormalities, and are identifiable by magnifying endoscopy, ACF should be appropriate chemopreventive targets in colorectal cancer. The advantages of using ACF as targets over using polyps are as follows: (1) short-term treatment for evaluation; (2) fewer complications caused by drugs; (3) good compliance. In fact, we have previously reported that the number of ACF is markedly reduced after treatment with sulindac for about 1 year [29]. Moreover, we preliminarily administered sulindac to patients with ACF for various periods (2–10 months) and examined the number of ACF before and after treatment; the results showed that most were eradicated within a few months. We are currently proceeding with a randomized, double-blind trial targeting ACF, which consists of a group receiving sulindac, a group receiving etodolac, and a group receiving a placebo for 2 months.

Overexpression of glutathione S-transferase P1-1 as a target molecule in human colon carcinogenesis

Glutathione S-transferase (GST) P1-1, a GST isozyme, is involved in detoxification [11, 19, 31]. GSTs are known to be cytoprotective enzymes whose functions

Table 1 Representative chemopreventive trials for colorectal cancer (*FAP* familial adenomatous polyposis)

Target	Sporadic/FAP	Agents	Duration	Result	Reference
Polyps	Sporadic	Sulindac	4 months	No change	10
	FAP	Sulindac	9 months	65% reduction	6
	FAP	Celecoxib	6 months	30% reduction	28
	FAP	Sulindac	4 months	No change	7
Incidence of polyps	Sporadic	Aspirin	1 year	37% reduction	26
	Sporadic	Aspirin	1–3 years	17% reduction	3
	Sporadic	Calcium	4 years	15% reduction	2
Incidence of cancer	Sporadic	Vitamin D	6 years	32% reduction	13
	Sporadic	Calcium	6 years	No change	13
	Sporadic	Vitamin D	4 years	26% reduction	5
	Sporadic	Calcium	4 years	No change	5
	Sporadic	Folate	6 years	31% reduction	8

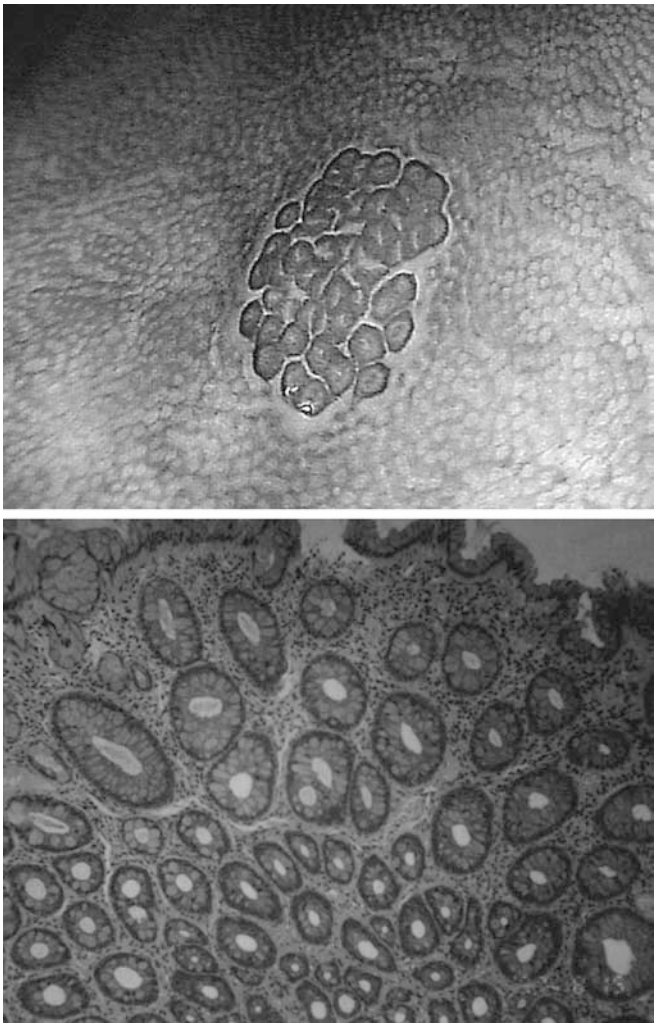

Fig. 1 Endoscopic and histological findings of aberrant crypt foci (ACF). Endoscopy with methylene blue staining reveals a small focus consisting of several crypts (ACF). Histological examination reveals slight enlargement, irregularity, and elongation of the crypts

include glutathione conjugation of xenobiotics, and binding of bile acids and NSAIDs. GSTP1-1 has been reported to be highly expressed in various cancers, including colon cancer [9, 14, 17]. During colon carcinogenesis, GSTP1-1 overexpression has been found not only in cancer, but also in polyps. We found GSTP1-1 overexpression in ACF as well as in adenoma and cancer. We also found that GSTP1-1 is coexpressed with mutated K-ras in ACF, adenoma, and cancers. By applying a transfection assay of various deletion mutants of GSTP1-1 gene and a gel shift assay, we showed that GSTP1-1 overexpression is induced by K-ras mutation through AP-1 activation [15].

Since GSTP1-1 sequestrates xenobiotics, such as bile salts, it is plausible that GSTP1-1 serves as a cytoprotective factor in ACF. To verify the role of GSTP1-1 in colon carcinogenesis, experiments using GSTP1-1 knockout mice were conducted. We treated GSTP1-1 knockout mice and control mice with azoxymethane and are currently analyzing their ACF and polyps.

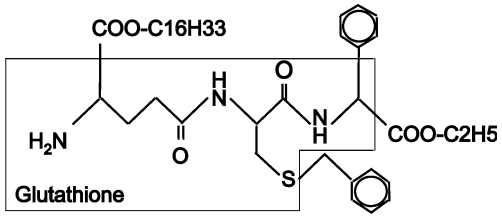

Fig. 2 Structure of GSTP1-1 specific inhibitor, γ-glutamyl S-benzylcysteinyl phenylglycyl ethylester hexadecylester. This inhibitor was synthesized by a conventional method for peptide synthesis

If GSTP1-1 is a cytoprotective factor in colorectal carcinogenesis, its inhibitor should inhibit colorectal carcinogenesis, and would be an effective chemopreventive agent. Recently we synthesized a GSTP1-1-specific inhibitor, γ-glutamyl S-benzylcysteinyl phenylglycyl ethylester hexadecylester from glutathione, a substrate of GST (Fig. 2) [16]. It was designed to release an active form, γ-glutamyl-S-benzylcysteinyl phenylglycine, when it enters cells and the ester bonds are cleaved by esterase. The active form has been proven to inhibit only GSTP1-1 activity with high specificity, but not the activity of other GST isozymes [12]. Currently, we are investigating the chemopreventive effect of this agent using a rat model of colorectal carcinogenesis. The data obtained so far show that the number of ACF is significantly reduced by the GSTP1-1 specific inhibitor. In the near future, the effects of the inhibitor on ACF and polyps will become evident.

References

1. Anderson WF, Umar A, Brawley OW (2003) Colorectal carcinoma in black and white race. Cancer Metastasis Rev 22:67
2. Baron JA, Beach M, Mandel JS, van Stolk RU, Haile RW, Sandler RS, Rothstein R, Summers RW, Snover DC, Beck GJ, Bond JH, Greenberg ER (1999) Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. N Engl J Med 340:101
3. Baron JA, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, McKeown-Eyssen G, Summers RW, Rothstein R, Burke CA, Snover DC, Church TR, Allen JI, Beach M, Beck GJ, Bond JH, Byers T, Greenberg ER, Mandel JS, Marcon N, Mott LA, Pearson L, Saibil F, van Stolk RU (2003) A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med 348:891
4. Bird RP (1987) Observation and qualification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. Cancer Lett 37:147
5. Bostick RM, Potter JD, Sellers TA, McKenzie DR, Kushi LH, Folsom AR (1993) Relation of calcium, vitamin D, and dairy food intake to incidence of colon cancer among older women. The Iowa Women's Health Study. Am J Epidemiol 137:1302
6. Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hylind LM, Celano P, Booker SV, Robinson CR, Offerhaus GJ (1993) Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med 328:1313
7. Giardiello FM, Yang VW, Hylind LM, Krush AJ, Petersen GM, Trimbath JD, Piantadosi S, Garrett E, Geiman DE, Hubbard W, Offerhaus GJ, Hamilton SR (2002) Primary chemoprevention of familial adenomatous polyposis with sulindac. N Engl J Med 346:1054

8. Giovannucci E, Stampfer MJ, Colditz GA, Rimm EB, Trichopoulos D, Rosner BA, Speizer FE, Willett WC (1993) Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Natl Cancer Inst 85:875
9. Kodate C, Fukushi A, Narita T, Kudo H, Soma Y, Sato K (1986) Human placental form of glutathione S-transferase (GST-pi) as a new immunohistochemical marker for human colonic carcinoma. Jpn J Cancer Res 77:226
10. Ladenheim J, Garcia G, Titzer D, Herzenberg H, Lavori P, Edson R, Omary MB (1995) Effect of sulindac on sporadic colonic polyps. Gastroenterology 108:1083
11. Listowsky I, Abramovitz M, Homma H, Niitsu Y (1988) Intracellular binding and transport of hormones and xenobiotics by glutathione-S-transferases. Drug Metab Rev 19:305
12. Lyttle MH, Hocker MD, Hui HC, Caldwell CG, Aaron DT, Engqvist-Goldstein A, Flatgaard JE, Bauer KE (1994) Isozyme-specific glutathione-S-transferase inhibitors: design and synthesis. J Med Chem 37:189
13. Martinez ME, Giovannucci EL, Colditz GA, Stampfer MJ, Hunter DJ, Speizer FE, Wing A, Willett WC (1996) Calcium, vitamin D, and the occurrence of colorectal cancer among women. J Natl Cancer Inst 88:1375
14. McKay JA, Murray GI, Weaver RJ, Ewen SW, Melvin WT, Burke MD (1993) Xenobiotic metabolising enzyme expression in colonic neoplasia. Gut 34:1234
15. Miyanishi K, Takayama T, Ohi M, Hayashi T, Nobuoka A, Nakajima T, Takimono R, Kogawa K, Kato J, Sakamaki S, Niitsu Y (2001) Glutathione transferase-π overexpression is closely associated with K-ras mutation during human colon carcinogenesis. Gastroenterology 121:865
16. Nakajima T, Takayama T, Miyanishi K, Nobuoka A, Thayashi T, Abe T, Kato J, Sakon K, Naniwa Y, Tanabe H, Niitsu Y (2003) Reversal of multiple drug resistance in cholangiocarcinoma by the GST-π specific inhibitor: O^1-hexadecyl γ-glutamyl-S-benzylcysteinyl-D-phenylglycine ethyl ester. J Pharmacol Exp Ther 306:861
17. Niitsu Y, Takahashi Y, Saito T, Hirata Y, Arisato N, Maruyama H, Kohgo Y, Listowsky I (1989) Serum glutathione-S-transferase-pi as a tumor marker for gastrointestinal malignancies. Cancer 63:317
18. Otori K, Konishi M, Sugiyama K, Hasebe T, Shimoda T, Kikuchi-Yanoshita R, Mukai K, Fukushima S, Miyaki M, Esumi H (1998) Infrequent somatic mutation of the adenomatous polyposis coli gene in aberrant crypt foci of human colon tissue. Cancer 83:896
19. Pickett CB, Lu AY (1989) Glutathione S-transferases: gene structure, regulation, and biological function. Annu Rev Biochem 58:743
20. Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, Vogelstein B, Kinzler KW (1992) APC mutations occur early during colorectal tumorigenesis. Nature 359:235
21. Pretlow TP, Barrow BJ, Ashton WS, O'Riordan MA, Pretlow TG (1991) Aberrant crypts: putative preneoplastic foci in human colonic mucosa. Cancer Res 51:1564
22. Pretlow TP, O'Riordan MA, Pretlow TG, Stellato TA (1992) Aberrant crypts in human colonic mucosa: putative preneoplastic lesions. J Cell Biochem [Suppl] 16G:55
23. Pretlow TP, Brasitus TA, Fulton NC, Cheyer C, Kaplan EL (1993) K-*ras* mutations in putative preneoplastic lesions in human colon. J Natl Cancer Inst 85:2004
24. Roncucci L, Stamp D, Medline A, Cullen JB, Bruce WR (1991) Identification and quantification of aberrant crypt foci and microadenomas in the human colon. Hum Pathol 22:287
25. Rougier P, Mitry E (2003) Epidemiology, treatment and chemoprevention in colorectal cancer. Ann Oncol 14 [Suppl 2]:ii3
26. Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, Petrelli N, Pipas JM, Karp DD, Loprinzi CL, Steinbach G, Schilsky R (2003) A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med 348:883
27. Smith AJ, Stern HS, Penner M, Hay K, Mitri A, Bapat BV, Gallinger S (1994) Somatic *APC* and K-*ras* codon 12 mutations in aberrant crypt foci from human colons. Cancer Res 54:5527
28. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK, Levin B (2000) The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 342:1946
29. Takayama T, Katsuki S, Takahashi Y, Ohi M, Nojiri S, Sakamaki S, Kato J, Kogawa K, Miyake H, Niitsu Y (1998) Aberrant crypt foci of the colon as precursors of adenoma and cancer. N Engl J Med 339:1277
30. Takayama T, Ohi M, Hayashi T, Miyanishi K, Nobuoka A, Nakajima T, Sato B, Takimoto R, Kato J, Sakamaki S, Niitsu Y (2001) Analysis of K-ras, APC and β-catenin in aberrant crypt foci in patients with adenoma and cancer, and familial adenomatous polyposis. Gastroenterology 121:599
31. Tsuchida S, Sato K (1992) Glutathione transferases and cancer. Crit Rev Biochem Mol Biol 27:337
32. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319:525
33. Yamashita N, Minamoto T, Ochiai A, Onda M, Esumi H (1995) Frequent and characteristic K-*ras* activation and absence of p53 protein accumulation in aberrant crypt foci of the colon. Gastroenterology 108:434