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Research Article

Investigation of the Prevalence and Number of Aberrant Crypt Foci Associated with Human Colorectal Neoplasm

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Abstract

Background: Aberrant crypt foci (ACF) are considered to be useful as surrogate biomarker for colorectal cancer (CRC), but the biological significance of ACF remains controversial. We attempted to investigate the relationship between the presence of ACF and human colorectal carcinogenesis using a relatively large sample size.

Methods: We carried out high-magnification chromoscopic colonoscopy to identify ACFs in 861 subjects undergoing a diagnostic endoscopy at the Yokohama City University Hospital. The present study compared the prevalence and number of ACFs in three subject groups (normal subjects, adenoma cases, and CRC cases). The correlations between the demographic and behavioral characteristics of the subjects and the prevalence of ACFs were also assessed.

Results: The prevalence of ACF was 64%, 88%, and 95%, and the mean number of ACF was 3.6, 6.2, and 10.1, in normal subjects, adenoma cases, and CRC cases, respectively. When differences in the prevalence and number of ACFs among age- and sex-stratified subject groups were examined, significant stepwise increments from normal subjects to adenoma cases to CRC cases were apparent ($P < 0.001$). Moreover, an age- and sex-adjusted multiple logistic regression analysis revealed that smoking and alcohol habits had a synergistic effect, increasing the prevalence of ACFs as well as the risk of CRC ($P < 0.001$).

Conclusions: These results suggested that ACF may serve as a reliable surrogate biomarker for human colorectal carcinogenesis.

Impact: The use of ACF as an endpoint may enable the size, duration, and cost of CRC chemoprevention studies to be reduced. *Cancer Epidemiol Biomarkers Prev; 20(9); 1918–24. ©2011 AACR.*

Introduction

Despite recent advances in therapeutic modalities, colorectal cancer (CRC) remains one of the most common causes of cancer-related death in developed countries (1). Currently, chemoprevention for CRC has attracted much attention. The purpose of chemoprevention is to reduce the future mortality of CRC using oral agents that can prevent the occurrence of cancer. Although the occurrence of CRC is the most reliable endpoint, such an endpoint is unsuitable for chemoprevention trials because the occurrence of CRC in the general population is relatively infrequent (1) and such trials would require long-term observation periods. Therefore, to evaluate the efficacy of chemopreventive agents in CRC chemoprevention trials, a more common surrogate biomarker that is robustly associated with CRC is required.

Colorectal carcinogenesis is based on the adenoma–carcinoma sequence, wherein adenomas, spurred by acquired genetic mutations, evolve into CRC. Adenomas have been established as premalignant lesions and are characterized by the presence of genetic and histologic changes. Endoscopic screening and the removal of adenomas can reduce the incidence of CRC by as much as 90% (2, 3). Despite retrospective and prospective studies supporting the use of adenomas as a surrogate biomarker of CRC in chemoprevention trials (4), the use of adenomas as a surrogate endpoint biomarker for CRC has some limitations. The most obvious limitation is that using adenoma formation as an efficacy endpoint requires hundreds of subjects and a very long observation period. Furthermore, to assess the effects of chemopreventive agents, the regression or loss of adenomas must be evaluated (5); therefore, a total colonoscopy is necessary. Unfortunately, these limitations result in poor compliance and a high frequency of dropouts over time, preventing a reasonable rate of

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progress for clinical research on CRC prevention. Moreover, large adenomas possibly contain cancer cells; therefore, the assessment of chemopreventive efficacy in patients with large adenomas would involve ethical problems. To overcome these problems, a more useful surrogate biomarker that is reliably correlated with the clinical response, that can be modulated by chemopreventive agents or behavioral characteristics (such as diet changes and smoking cessation) within a short period of time, and that is relatively simple to measure is needed.

Aberrant crypt foci (ACF) were discovered as the earliest microscopic lesions to appear in the colonic mucosa of mice treated with azoxymethane (6). Many studies have shown a dose–response relationship between carcinogens, such as azoxymethane and dimethylhydrazine, and the number of ACF induced (7–11). Moreover, in recent studies, numerous chemopreventive agents have been shown to reduce the number of ACFs in animal models of chemical colonic carcinogenesis. Importantly, many agents that block ACF growth were also shown to prevent tumor development in these carcinogen-treated rodent models (11). Thus, in rodent models, ACFs have been established as a precursor of CRC. Shortly after such descriptions in rodent models were made, ACFs were discovered in pathologic specimens of human colonic mucosa (12–14). ACFs were subsequently identified in the colonic mucosa *in vivo* using high-magnification chromoscopic colonoscopy (HMCC) with methylene blue staining (15). Although several previous epidemiologic studies have revealed significant associations between the prevalence and/or number of ACFs and the synchronous presence of advanced neoplasms, including both adenoma and CRC (15–22), most of the sample sizes in these studies were relatively small. Consequently, the findings were somewhat conflicting. In addition, these studies had limited data about other personal characteristics, such as smoking habit, alcohol habit, and obesity—all of which are related to an increased risk of CRC. If ACFs are indeed a surrogate biomarker for CRC, the epidemiology of ACFs would likely be similar to that of CRC. Therefore, we attempted to investigate the relationship between the presence of ACFs and colorectal carcinogenesis using a larger sample size. Here, we compared the prevalence and number of ACFs in 3 subject groups (normal subjects, adenoma cases, and CRC cases). Moreover, we evaluated the association between the presence of ACFs and the adenoma history. The correlations between the demographic and behavioral characteristics in relation to colorectal carcinogenesis and the prevalence and number of ACFs were also assessed. Our results may help to further evaluations of the potential utility of ACF as a surrogate biomarker for CRC.

Materials and Methods

Subjects

The study protocol was approved by the Yokohama City University Hospital Ethics Committee. Between 2004 and 2009, we enrolled 861 subjects who underwent diagnostic endoscopy at the Yokohama City University Hospital, Japan: of the 861 subjects, 383 had no apparent lesions of the colorectum on colonoscopy (normal subjects), 372 had colorectal adenoma(s), and 106 had CRC. Subjects were excluded if they had undergone previous surgical or endoscopic excision of colonic adenomas and/or cancer or if they had familial adenomatous polyposis, inflammatory bowel disease, or radiation colitis. Written informed consent was obtained from all the subjects prior to their participation in the study. Data on the demographic and behavioral characteristics of the subjects pertaining to the risk of the development of CRC, including smoking habit, alcohol habit, and body mass index (BMI), were obtained from the subjects prior to the performance of the colonoscopy.

HMCC

A Fujinon EC-490ZW5/M colonoscope was used for the magnifying colonoscopy (Fujinon Toshiba ES Systems Co., Ltd.). All the subjects were subjected to bowel preparation using a polyethylene glycol–based solution and underwent a total colonoscopy before rectal ACF imaging. Any detected adenomas were biopsied and the histopathologic appearance was analyzed. Advanced adenoma was defined as an adenoma lesion measuring 1 cm or greater in diameter and/or exhibiting a villous histology and/or high-grade dysplasia. Subsequently, 0.25% methylene blue was applied to the mucosa using a spray catheter. On the basis of the results of a previous study, the ACFs were counted in the lower rectal region, from the middle Houston valve to the dentate line (15). To guard against double counting, the ACFs were counted in a sequential fashion during a single withdrawal of the endoscope. We evaluated the presence of ACFs and the category of the subject (normal subjects, adenoma cases, and CRC cases) simultaneously.

Criteria used for the endoscopic diagnosis

ACFs were defined as lesions in which the crypts were larger in diameter and showed a darker staining with methylene blue than normal crypts, often with oval or slit-like lumens and a thicker epithelial lining (ref. 15; Fig. 1).

Statistical analysis

Data were expressed as the mean ± SD for continuous variables and as a proportion (%) for categorical variables. The prevalence of ACFs among the normal subjects, adenoma cases, and CRC cases were compared using age- and sex-adjusted logistic regression analyses. The numbers of ACFs among these 3 groups were also compared using the Kruskal–Wallis test or an age- and sex-adjusted linear regression analysis. The χ^2 test and the Mann–Whitney U test were used to investigate the association between the presence of ACFs and the adenoma status as well as the association between the presence of ACFs and the location of adenoma(s)/CRC. In addition, univariate and multivariate logistic regression

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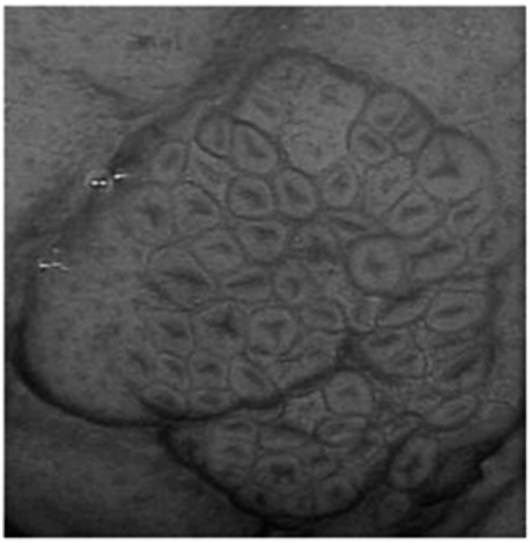

Figure 1. Typical endoscopic appearance of human ACF. This photograph was obtained using a Fujinon EC-490ZW5/M colonoscope after the rectal mucosa had been stained with 0.2% methylene blue.

analyses were used to identify variables with significant independent effects on the prevalence of ACFs among normal subjects. Univariate and multivariate linear regression analyses were also conducted to identify significant variables influencing the number of ACFs. The variables entered in the model included age, sex, smoking habit, alcohol habit, and BMI. Unless otherwise specified, a value of $P < 0.05$ was considered statistically significant. All the analyses were conducted using the SPSS statistical package (version 11.0 for Mac OS X).

Results

Characteristics of the subjects

The characteristics of the subjects according to study group (normal subjects, adenoma cases, and CRC cases) are shown in Table 1. The subjects ranged in age from 19 to 89 years (62.2 ± 12.4): normal subjects, 19 to 85 years (59.3 ± 13.8); adenoma cases, 31 to 88 years (64.2 ± 10.9); and CRC cases, 34 to 89 years (65.6 ± 9.4). A total of 4,742 ACFs were visualized endoscopically in 861 subjects: 1,382 in normal subjects, 2,288 in the adenoma cases, and 1,072 in the CRC cases. The prevalence of ACFs was 64%, 88%, and 95% for the normal subjects, adenoma cases, and CRC cases, respectively. The mean number of ACFs was 3.6 ± 5.2, 6.2 ± 7.0, and 10.1 ± 7.9 in the normal subjects, adenoma cases, and CRC cases, respectively.

Prevalence and number of ACF in the three subject groups stratified according to age and sex

The prevalence and number of ACFs in the 3 subject groups according to age and sex are shown in Table 2. The prevalence of ACF was as high as 51% to 74% even in normal subjects. The prevalence of ACFs in the CRC cases was as high as 91% to 100%, whereas that in the adenoma cases was intermediate. An age-adjusted logistic regression analysis for the 3 subject groups stratified according to sex showed that the differences of the prevalence of ACFs among the 3 subject groups (normal subjects, adenoma cases, and CRC cases) were significant ($P < 0.001$ and $P < 0.001$ for men and women, respectively). In addition, an age-adjusted linear regression analysis for the 3 subject groups stratified according to sex showed that the differences of the number of ACF among the 3 subject groups (normal subjects, adenoma cases, and CRC cases) were significant ($P < 0.001$ and $P < 0.001$ for men and women, respectively).

Differences in the presence of ACFs between subjects with nonadvanced and advanced adenomas

The relationship between the presence of ACFs and the adenoma history is shown in Table 3. The prevalence of ACFs in subjects with advanced adenoma(s) did not differ significantly from that of subjects with nonadvanced adenoma(s) (89% and 87%, respectively; $P = 0.41$). However, the number of ACFs in subjects with

Table 1. Characteristics of the subjects

	Normal subjects	Adenoma cases	CRC cases
Number of subjects	383	372	106
Age, y			
Mean ± SD	59.3 ± 13.8	64.2 ± 10.9	65.6 ± 9.4
Median	62	64.5	66
Sex (M/F)	211/172	265/107	73/33
Number of subjects with ACF	246	326	101
ACF prevalence, %	64	88	95
Total number of ACF	1,382	2,288	1,072
ACF number, mean ± SD	3.6 ± 5.2	6.2 ± 7.0	10.1 ± 7.9

NOTE: Normal subjects were defined as subjects with no apparent lesions of the colorectum on total colonoscopy.

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Table 2. Prevalence and number of ACFs among the 3 subject groups stratified according to age and sex

	Male				Female			
	<60 y	60–69 y	≥70 y	Total	<60 y	60–69 y	≥70 y	Total
ACF prevalence[a]								
Normal subjects								
Number of subjects	89	62	60	211	78	52	42	172
Prevalence, %	62	74	67	67	51	71	67	61
Adenoma cases								
Number of subjects	79	92	94	265	29	44	34	107
Prevalence, %	89	88	95	91	76	82	82	80
CRC cases								
Number of subjects	15	34	24	73	9	11	13	33
Prevalence, %	93	94	96	95	100	91	100	97
ACF number,[b] *mean ± SD*								
Normal subjects	2.6 ± 3.2	5.3 ± 7.1	4.6 ± 6.2	3.9 ± 5.6	1.8 ± 2.6	3.8 ± 5.0	5.0 ± 6.5	3.2 ± 4.7
Adenoma cases	5.0 ± 5.5	6.8 ± 7.9	8.1 ± 8.0	6.7 ± 7.4	4.7 ± 5.9	3.8 ± 4.1	6.1 ± 7.8	4.8 ± 6.0
CRC cases	8.7 ± 8.4	10.5 ± 7.4	10.0 ± 7.3	10.0 ± 7.5	10.9 ± 11.6	8.5 ± 9.3	11.8 ± 6.0	10.5 ± 8.7
P	<0.001	<0.001	<0.001	<0.001‡	<0.001	0.19	<0.005	<0.001[c]

[a]$P < 0.001$ and $P < 0.001$ (men and women, respectively), calculated using an age-adjusted logistic regression analysis for the 3 subject groups stratified according to sex.
[b]Differences among the age-stratified subject groups (<60, 60–69, and ≥70 years) were analyzed using the Kruskal–Wallis test.
[c]An age-adjusted linear regression analysis was conducted to evaluate the differences among the 3 subject groups stratified according to sex.

advanced adenoma(s) was larger than that in subjects with nonadvanced adenoma(s) (7.8 ± 8.2 and 5.1 ± 6.0, respectively; $P < 0.005$).

Relationship between the presence of ACFs and the location of adenoma/CRC

The relationship between the presence of ACFs and the location of adenoma/CRC is shown in Table 4. Sixty-eight of the 372 adenoma cases (18%) had adenoma(s) only in the proximal colon. No significant differences were observed between the prevalence and number of ACFs and the location of the adenoma(s) ($P = 0.86$ and $P = 0.73$, respectively). Twenty-nine of the 102 CRC cases (28%) had proximal CRC. No significant differences were observed between the prevalence and number of ACFs and the location of the CRC ($P = 0.52$ and $P = 0.26$, respectively).

Correlations between the presence of ACFs and demographic and behavioral characteristics pertaining to the risk of colorectal carcinogenesis

To investigate the risk factors for the prevalence of ACF, univariate and multivariate logistic regression analyses were conducted in normal subjects (Table 5). We defined smoking habit as positive for subjects with more than 10 pack-years who were still smoking or who had quit within the past 10 years; alcohol habit was defined as positive for subjects with alcohol consumption in excess

Table 3. Differences in the presence of ACFs between subjects with nonadvanced and advanced adenoma(s)

	N	ACF prevalence,[a] %	ACF number,[b] mean ± SD
Nonadvanced adenoma	230	87	5.1 ± 6.0
Advanced adenoma	142	89	7.8 ± 8.2
P		0.41	<0.005

NOTE: Advanced adenoma was defined as an adenoma lesion measuring 1 cm or greater in size and/or exhibiting a villous histology and/or high-grade dysplasia.
[a]*P* values were calculated using the χ^2 test.
[b]*P* values were calculated using the Mann–Whitney *U* test.

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Table 4. Relationship between the presence of ACFs and the location of adenoma/CRC

Location	*N*	ACF prevalence,[a] %	ACF number,[b] mean ± SD
Adenoma cases			
Including distal colon	304	88	6.1 ± 6.9
Only in proximal colon	68	88	6.4 ± 7.6
P		0.87	0.73
CRC cases			
Distal colon	77	96	10.4 ± 7.6
Proximal colon	29	93	9.3 ± 8.6
P		0.52	0.26

NOTE: The distal colon was defined as the region of colonic lesion from the splenic flexure to the dentate line. The proximal colon was defined as the region of colonic lesion from the cecum to the splenic flexure.

[a]*P* values were calculated using the χ^2 test.

[b]*P* values were calculated using the Mann–Whitney *U* test.

of 45 g/d. Both of these factors are reported to associate with an increased risk of adenoma and CRC (23–28). Age- and sex-adjusted multivariate analyses revealed that smoking habit [odds ratio (OR) = 1.6; 95% CI = 0.9–3.1] and alcohol habit (OR = 2.0; 95% CI = 0.8–5.0) were not independent risk factor for the prevalence of ACFs ($P = 0.12$ and $P = 0.15$, respectively); however these 2 factors (OR = 5.4; 95% CI = 2.3–13.0) synergistically increased the prevalence of ACFs ($P < 0.001$). Obesity was also reported to associate with an increased risk of adenoma and CRC (28). We defined obesity as positive for subjects with a BMI of 25 or grater. Obesity (OR = 1.5; 95% CI = 0.8–2.6) was also not an independent risk factor for the prevalence of ACFs ($P = 0.17$). We also conducted univariate and age- and sex-adjusted multivariate linear regression analyses to evaluate the correlations between the number of ACFs and these factors. Smoking and alcohol habits also synergistically increased the number of ACFs, but this trend was only borderline significant ($P = 0.06$; Supplementary Table S1).

Discussion

In our study, significant stepwise increments in both the prevalence and the number of ACFs were observed from normal subjects to adenoma cases to CRC cases. In addition, the mean number of ACF was significantly higher in the subject group with advanced adenoma than in the subject group with nonadvanced adenoma. These results indicate that ACF may serve as a reliable surrogate biomarker of human colorectal carcinogenesis.

Most previous studies (15–18, 20–22) have evaluated ACF in the lower rectal region because HMCC is technically easier to conduct at this location, is suitable for use as a follow-up examination, and is well tolerated by patients. Therefore, we evaluated the ACFs in the lower rectal region, similar to previous studies. To evaluate whether the rectal ACF reflects the total colonic adenoma/CRC, we examined associations between the presence of rectal ACFs and the locations of the adenoma/CRC. In our study, no significant differences were observed between the prevalence and the number of ACFs in subjects who had only proximal colonic adenoma/CRC and subjects who had at least 1 distal colonic adenoma/CRC. This result indicates that rectal ACF examinations may be useful as a biomarker not only for distal colonic neoplasia but also for proximal colonic neoplasia.

The development of CRC is influenced by several acquired risk factors including dietary factors and lifestyle factors. If ACFs are indeed a surrogate biomarker of CRC, then their epidemiology is likely to be similar to that of CRC. If risk factors influence colorectal carcinogenesis at an early stage, then they may also be associated with the formation of ACFs. Therefore, we evaluated whether risk factors which associate with the development of CRC were independently associated with the presence of ACFs in normal subjects. In our study, smoking habit

Table 5. Age- and sex-adjusted multiple logistic regression analysis of behavioral characteristics and the prevalence of ACFs in normal subjects

Variable	Proportion, %	ACF prevalence, %	OR (95% CI)			
			Univariate	*P*	Multivariate	*P*
Smoking (−), alcohol (−)	63	58	1 (reference)	–	1 (reference)	–
Smoking (+), alcohol (−)	16	67	1.5 (0.8–2.7)	0.17	1.6 (0.9–3.1)	0.12
Smoking (−), alcohol (+)	7	73	2.0 (0.8–4.9)	0.13	2.0 (0.8–5.0)	0.15
Smoking (+), alcohol (+)	14	87	4.8 (2.1–11.1)	<0.001	5.4 (2.3–13.0)	<0.001

NOTE: Smoking habit was defined as positive if the subject had more than 10 pack-years and was still smoking or had quit within the past 10 years. Alcohol habit was defined as positive if the subject's alcohol consumption exceeded 45 g/d. The multivariate logistic regression analysis was adjusted for age and sex.

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and alcohol habit synergistically increased the prevalence of ACFs in a significant manner. Interestingly, recent studies have revealed that cigarette smoking and heavy alcohol intake also interact in an additive manner, increasing the risk of CRC, similar to results seen in the aerodigestive tract (29, 30). Tobacco contains a large number of carcinogens that may bind to DNA and form adducts, potentially causing irreversible genetic damage to the normal colonic mucosa (31). On the other hand, alcohol is metabolized to acetaldehyde, which binds to DNA and forms carcinogenic adducts (32). Therefore, these 2 factors may share a common pathway in promoting colorectal carcinogenesis at an early stage and initiating ACF formation. On the other hand, obesity was not strongly associated with the prevalence of ACFs because only a few patients were regarded as obese in our study. In contrast to our hypothesis, no significant associations were observed between the number of ACFs and these factors, although smoking and alcohol habits tended to increase the number of ACFs in a synergistic manner. A not insignificant number of subjects exhibited an extremely high density of ACFs (as high as 30), even in normal subjects; therefore, the wide variance in the number of ACFs might have extinguished the statistical significance (Supplementary Fig. S1).

Although most previous epidemiologic studies of ACFs have shown a significant correlation between the presence of ACFs and synchronous advanced neoplasia (15–22), a recent multicenter study raised serious questions about whether ACFs can be used as a surrogate biomarker for CRC (33). However, their subject groups were determined 8 years, on average, prior to the actual ACF examination. In addition, they determined the subject group on the basis of the results of flexible sigmoidoscopy; thus, proximal adenomas may have been missed. These facts suggest that their control group may have contained a not insignificant number of subjects with adenoma. Therefore, their study may not actually show an association between the presence of ACFs and the adenoma status. However, such considerations are inadequate to explain this discrepancy. Differences in participant characteristics, such as race, age and behavioral factors, may be associated with this discrepancy. Variations in the criteria used to detect ACFs and the method used to visualize ACFs may also affect this discrepancy. A large prospective and cross-sectional study would be useful for resolving this discrepancy.

Recently, several prospective studies have been conducted using the presence of ACF as a surrogate biomarker for CRC in chemoprevention trials in humans (34–36). ACFs are considered to be a heterogeneous group of lesions, some, but not all, of which may be robustly associated with the risk of CRC, as the prevalence of ACF was as high as 70% even in normal subjects. Interestingly, our results suggested that even if a very small subset or none of the ACFs may progress to CRC, ACF may still be useful as a surrogate biomarker for CRC. In humans, Shpitz and colleagues showed that the proliferating cell nuclear antigen (PCNA) labeling indices for ACFs were significantly higher than those for normal mucosa (14). In addition, we previously showed that metformin, which inhibits the mTOR pathway through the activation of AMPK, suppresses cellular proliferation and ACF formation (35). These results suggested that ACF may be a marker for epithelial proliferation. Importantly, previous studies have showed that a high proliferative activity in the colon mucosa is associated with an increased risk of CRC (37).

In conclusion, we confirmed that the prevalence and mean number of ACFs significantly increased with the stage of the adenoma–carcinoma sequence using age- and sex-adjusted analyses of a relatively large sample. We also showed that smoking and alcohol habits synergistically increased the prevalence of ACFs as well as the risk of CRC. These results suggested that ACFs may be useful as a reliable surrogate biomarker for human colorectal carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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