Association between factors associated with colorectal cancer and rectal aberrant crypt foci in humans

AKIKO EZUKA[1], EIJI SAKAI[2], KENICHI KAWANA[1], HAJIME NAGASE[1], YUKIO KAKUTA[3], SHIORI UCHIYAMA[2], HIDENORI OHKUBO[2], TAKUMA HIGURASHI[2], TAKASHI NONAKA[2], HIROKI ENDO[2], HIROKAZU TAKAHASHI[2,4] and ATSUSHI NAKAJIMA[2]

[1]Department of Gastroenterology, Yokohama Rosai Hospital, Yokohama, Kanagawa 222-0036; [2]Department of Gastroenterology and Hepatology, Yokohama University School of Medicine, Yokohama, Kanagawa 236-0004; [3]Department of Pathology, Yokohama Rosai Hospital, Yokohama, Kanagawa 222-0036; [4]Department of Gastroenterology, National Cancer Center, Tokyo 104-0045, Japan

Received October 11, 2014; Accepted July 28, 2015

DOI: 10.3892/ol.2015.3763

Abstract. Aberrant crypt foci (ACF) are regarded as potential biomarkers for colorectal cancer (CRC), and have been used as such in recent early-phase chemoprevention trials. However, the associations between the presence of ACF and other factors associated with the development of CRC, such as lifestyle factors, medication use and comorbid medical conditions, remain unknown. Thus, the present retrospective, large, cross-sectional study was conducted to evaluate the potential usefulness of ACF as a surrogate biomarker of CRC. Total colonoscopy was performed and the number of rectal ACF was counted in a total of 902 subjects. A retrospective review of the medical records of the study subjects was performed, and the factors associated with the increased prevalence of ACF was investigated using univariate and multivariate logistic regression analyses. The analysis results identified older age [odds ratio (OR), 9.24; 95% confidence interval (CI), 4.80-17.8; P<0.01], smoking habit (OR, 1.78; 95% CI, 1.20-2.63; P<0.01) and use of insulin (OR, 9.97; 95% CI, 1.28-77.5; P=0.03) as significant independent risk factors associated with the increased prevalence of ACF, regardless of the presence/absence of colon tumors. In addition, it was revealed that the prevalence and number of ACF, and the Ki-67 labeling indices of the colonic epithelial cells were significantly higher in diabetic patients receiving insulin therapy than in those not receiving insulin therapy (P<0.01, P=0.03 and P=0.01, respectively). In conclusion, the potential usefulness of ACF as a surrogate biomarker of CRC was confirmed, although useful data could not be obtained on candidate chemopreventive agents. These results indicated that insulin can enhance colonic epithelial proliferative activity and induce the formation of ACF, thereby possibly triggering CRC development.

Correspondence to: Professor Atsushi Nakajima, Department of Gastroenterology and Hepatology, Yokohama University School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan
E-mail: nakajima-tky@umin.ac.jp

Abbreviations: CRC, colorectal cancer; NSAIDs, non-steroidal anti-inflammatory drugs; EPA, eicosapentaenoic acid; PPARγ, peroxisome proliferator-activated receptor γ; ACF, aberrant crypt foci; BMI, body mass index; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval

Key words: colorectal cancer, aberrant crypt foci, colorectal carcinogenesis, insulin, Ki-67

Introduction

Colorectal cancer (CRC) is one of the most common causes of cancer-related mortality in the world, and its prevalence and mortality rates have been increasing (1). Currently, chemoprevention, a strategy which attempts to prevent cancer using non-toxic chemical entities, has attracted much attention (2,3). Although the possible effects of >50 agents, including non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, metformin, fiber, statins, eicosapentaenoic acid (EPA), peroxisome proliferator-activated receptor γ (PPARγ) agonists and folate, have been evaluated for the protection against CRC development in epidemiological studies (4-6), the use of the majority of these agents is only in the early experimental stage. Although the occurrence of CRC and/or colorectal adenoma is regarded as a reliable endpoint in chemoprevention trials (7), evaluation of such an endpoint would require inclusion of hundreds of subjects in the studies and extremely long observation periods. To maximize the efficiency of early-phase chemoprevention trials, more easily modifiable and easy-to-measure surrogate biomarkers are therefore required.

Colorectal carcinogenesis is based on the adenoma-carcinoma sequence; CRC is believed to develop through the accumulation of multiple acquired genetic and epigenetic alterations that cause the malignant transformation of the normal colonic epithelium to adenocarcinoma (8,9). Over the last decade, the emergence of aberrant crypt foci (ACF) as putative precursors to colorectal adenoma has been

witnessed, and these ACF have come to be regarded as potential biomarkers for CRC (10-18). In the rodent model, the formation of ACF has been demonstrated to be enhanced by cancer promoters and suppressed by chemopreventive agents (19-23). These results indicate that chemopreventive agents against CRC may also prevent the formation of ACF, not only in carcinogen-treated rodent models, but also in humans. However, few studies have investigated the correlations between the presence of ACF and the use of candidate chemopreventive agents of CRC in humans. Moreover, the associations between the presence of ACF and lifestyle factors, including obesity, smoking and alcohol intake) (24,25), or comorbid medical conditions, such as diabetes mellitus (DM) (26), considered to be associated with CRC development remain unknown.

In the present study, the association between the presence of ACF and lifestyle factors or comorbid medical conditions was investigated. Furthermore, the correlation between the presence of ACF and the use of medications that are believed to be associated with colorectal carcinogenesis are also investigated. If ACF were indeed a reliable biomarker of CRC in humans, their formation may also be expected to be closely associated with the aforementioned factors. The present results may pave the way for further evaluations of the potential usefulness of ACF as a surrogate biomarker of CRC, and also for further evaluation of candidate chemopreventive agents.

Materials and methods

Patients. The medical records of 1,024 patients who underwent total colonoscopy and rectal ACF counting at the Yokohama City University Hospital (Yokohama, Kanagawa, Japan) between May 2004 and October 2013 were reviewed retrospectively. The collected patient data included the age, gender, body mass index, smoking history, alcohol intake history, history of medication use (NSAIDs, aspirin, statins, metformin, EPA, PPARγ agonists and insulin), and history of comorbid medical conditions (hypertension, hyperlipidemia and DM). Subjects were excluded if they had a history of familial adenomatous polyposis, hereditary non-polyposis CRC, inflammatory bowel disease or radiation colitis, or had undergone a prior large-bowel resection. Finally, a total of 902 subjects were enrolled in this study. To confirm the close association between the presence of ACF and colonic tumors, the subjects were divided into three categories (normal subjects, adenoma cases and CRC cases), as previously described (15). This study was conducted with the approval of the Yokohama City University Hospital Ethics Committee. Written informed consent was obtained from all the subjects prior to their participation in the study.

Endoscopic identification of ACF. ACF are usually identified as clusters of crypts that stain darker than the surrounding normal mucosa. Crypts with a larger size, a raised appearance, a thicker epithelial lining, dilated or slit-like crypt lumina, and an increased pericryptal area as compared to the surrounding normal mucosa are the most frequently used criteria to identify ACF (18). A Fujinon EC-490ZW5/M magnifying colonoscope (Fujifilm Medical Co., Ltd., Tokyo, Japan) was used for identifying the ACF. All the subjects underwent bowel preparation using a polyethylene glycol-based solution and total colonoscopy prior to the rectal ACF imaging. Subsequently, 0.25% methylene blue was applied to the mucosa using a spray catheter and allowed to stand for 2 min. ACF counting was performed in the lower rectal region, from the middle Houston valve to the dentate line.

Cell proliferative activity of the rectal colonic epithelial mucosa. To evaluate the association between the colonic epithelial mucosal proliferative activity and the presence of ACF, the Ki-67 labeling index was calculated. In certain cases, normal-appearing rectal mucosa was biopsied, fixed with formalin and embedded in paraffin, and 4-μm thick sections were prepared. Immunohistochemical staining for Ki-67 with anti-Ki-67 rabbit monoclonal antibody (Nichirei Bioscience Inc., Tokyo, Japan) was performed as previously described (27).

Statistical analysis. The differences in the prevalence and number of ACF among the three groups (normal subjects, adenoma cases and CRC cases) were compared using the χ^2 (or Fisher's exact) test or the Kruskal-Wallis test. The associations between the presence of ACF and lifestyle factors, history of use of oral medications or comorbid medical conditions were evaluated by the χ^2 (or Fisher's exact) test and/or Student's t-test. To identify the risk factors for the increased prevalence of ACF, univariate and multivariate logistic regression analyses were performed. The differences in the Ki-67 labeling indices were evaluated by the Mann-Whitney U test. Unless otherwise specified, $P<0.05$ was considered to denote a statistically significant difference. All analyses were performed using the SPSS statistical software package (version 11.0; SPSS, Inc., Chicago, IL, USA).

Results

Association between the presence of ACF and colorectal tumors. The prevalence and number of ACF in each of the three study groups (normal subjects, adenoma cases and CRC cases) are shown in Table I. The prevalence/number of ACF were 66.1%/5.3±4.8, 84.0%/5.3±4.8 and 88.9%/7.3±5.7, in the normal subjects, adenoma cases and CRC cases, respectively. Consistent with previous studies (15,18), the prevalence and number of ACF increased significantly from the normal subjects to the CRC cases ($P<0.01$ and $P<0.01$, respectively).

Associations between the presence of ACF and clinical factors that are believed to be associated with colorectal carcinogenesis. The associations between the presence of ACF and lifestyle factors, use of oral medications or comorbid medical conditions are shown in Table II. The prevalence of ACF was significantly higher in patients with an older age (69.3 vs. 95.6%; $P<0.01$), a male gender (70.9 vs. 79.9%; $P<0.01$), a positive smoking habit (69.3 vs. 85.4%; $P<0.01$), positive alcohol intake (72.3 vs. 81.0%; $P<0.01$), DM (74.3 vs. 84.8%; $P<0.01$) and in those receiving insulin therapy (75.5 vs. 97.8%; $P<0.01$) than in those without these

Table I. Prevalence and number of ACF in the study groups.

Factor	Normal	Adenoma cases	CRC cases	P-value
Number of patients	391	412	99	
ACF prevalence (%)[a]	66.1	84.0	88.9	<0.001
ACF number (mean ± SD)[b]	5.3±4.8	5.6±5.3	7.3±5.7	<0.001

[a]P-value calculated by the χ^2 test. [b]P-value calculated by the Kruskal Wallis test. ACF, aberrant crypt foci; CRC, colorectal cancer; SD, standard deviation.

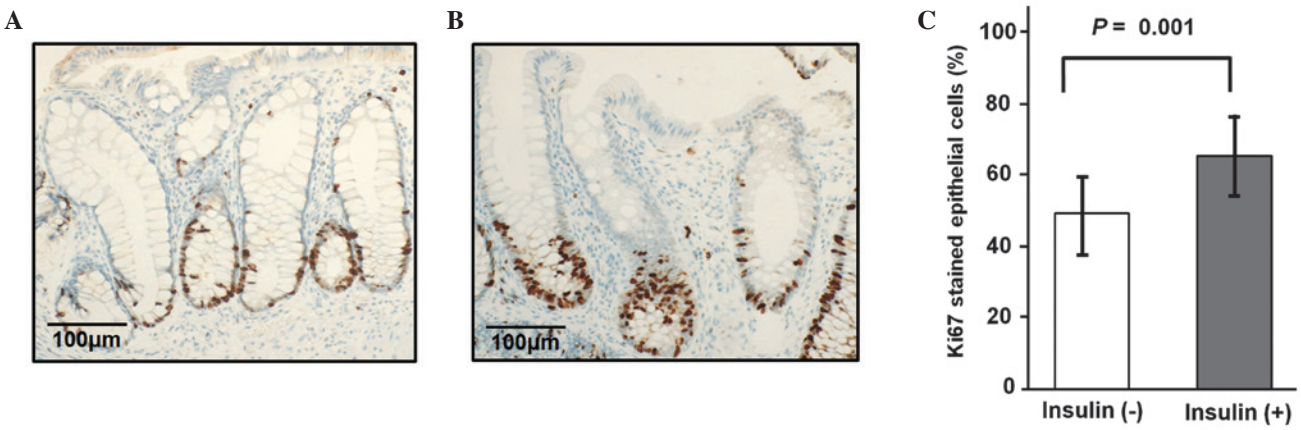

Figure 1. Immunohistochemical staining and Ki-67 labeling indices for normal-appearing rectal mucosa in diabetic patients. Immunohistochemical staining of Ki-67 in biopsy specimens of the rectal epithelium obtained from diabetic patients (A) not receiving therapy and (B) receiving insulin therapy. (C) The Ki-67 labeling index was significantly higher in the epithelium obtained from the diabetic patients receiving insulin therapy compared with that in those not receiving insulin (64.1 vs. 48.6%; P=0.01).

factors. ACF were present in higher numbers in patients with an older age (2.7±3.9 vs. 8.6±5.5; P<0.01), a male gender (3.9±5.1 vs. 4.7±5.2; P=0.03), a positive smoking habit (3.7±5.0 vs. 5.2±5.3; P<0.01), DM (4.1±5.0 vs. 5.3±5.7; P=0.04) and in those receiving insulin therapy (4.3±5.1 vs. 6.4±6.2; P<0.01) than in those without these factors. However, a lower prevalence and number of ACF were not significantly associated with any of the examined factors.

Risk factors for the increased prevalence of ACF. To gain insight into the contribution of the increased prevalence of ACF, univariate and multivariate logistic regression analyses were performed, as shown in Table III. Univariate analysis identified an older age [odds ratio (OR), 9.68; 95% confidence interval (CI), 5.17-18.1; P<0.01], the male gender (OR, 1.63; 95% CI, 1.19-2.23; P<0.01), the presence of adenoma (OR, 2.65; 95% CI, 1.90-3.71; P<0.01), the presence of CRC (OR, 4.12; 95% CI, 2.13-7.99; P<0.01), a history of smoking (OR, 2.56; 95% CI, 1.83-3.58; P<0.01), a history of alcohol intake (OR, 1.63; 95% CI, 1.19-2.23; P<0.01), the presence of DM (OR, 1.92; 95% CI, 1.26-2.94; P<0.01), a history of insulin therapy (OR, 14.6; 95% CI, 2.00-106.8; P<0.01) as significant factors associated with the increased prevalence of ACF. Furthermore, age- and gender-adjusted multivariate logistic regression analysis identified a history of smoking (OR, 1.78; 95% CI, 1.20-2.63; P<0.01) and a history of insulin therapy (OR, 9.97; 95% CI 1.28-77.5; P=0.03) as showing a significant independent association with an increased prevalence of ACF, regardless of the presence/absence of colonic tumors.

Comparison of the presence of ACF between diabetic patients receiving and not receiving insulin therapy. To investigate the difference in the prevalence of ACF between diabetic patients receiving and not receiving insulin therapy, the prevalence and number of ACF were compared between 46 diabetic patients receiving insulin therapy and 151 diabetic patients not receiving insulin therapy (Table IV). The prevalence of ACF in the diabetic patients receiving insulin therapy was significantly higher than that in those not receiving insulin therapy (97.8 vs. 80.1%; P<0.01). The median number of ACF in the diabetic patients receiving insulin therapy was significantly higher than that in those not receiving insulin therapy (4.5±2.5 vs. 3.0±3.0; P=0.03).

Comparison of the proliferative activity of the colonic mucosal epithelium in diabetic patients receiving and not receiving insulin therapy. The Ki-67 data from a total of 20 biopsy samples of normal-appearing rectal mucosa obtained from diabetic patients (including 10 patients receiving insulin and 10 patients not receiving insulin) were measured. The differences in the immunohistochemical staining and labeling index for Ki-67 between the specimens obtained from the diabetic patients receiving and not receiving insulin therapy are shown in Fig. 1. The proportion of proliferating cells in the patients receiving insulin therapy was significantly

Table II. Association between the presence of ACF and clinical factors believed to be associated with colorectal carcinogenesis.

Factor	Variable (-)	Variable (+)	P-value
Demographic and lifestyle factors			
Older age >70 years			
ACF prevalence, n (%)	451 (69.3)	240 (95.6)	<0.01
ACF number (mean ± SD)	2.7±3.9	8.6±5.6	<0.01
Male gender			
ACF prevalence, n (%)	234 (70.9)	457 (79.9)	<0.01
ACF number (mean ± SD)	3.9±5.1	4.7±5.2	0.03
BMI ≥25			
ACF prevalence, n (%)	512 (75.7)	179 (79.2)	0.27
ACF number (mean ± SD)	5.3±6.8	6.2±6.6	0.08
Smoking habit			
ACF prevalence, n (%)	341 (69.3)	350 (85.4)	<0.01
ACF number (mean ± SD)	3.7±5.0	5.2±5.3	<0.01
Alcohol intake			
ACF prevalence, n (%)	329 (72.3)	362 (81.0)	<0.01
ACF number (mean ± SD)	4.3±5.6	4.4±4.6	0.84
Comorbid medical conditions			
Hypertension			
ACF prevalence, n (%)	443 (75.3)	248 (79.0)	0.25
ACF number (mean ± SD)	4.4±4.9	4.8±5.5	0.08
Hyperlipidemia			
ACF prevalence, n (%)	555 (76.3)	136 (77.7)	0.77
ACF number (mean ± SD)	4.5±4.9	4.9±5.9	0.06
DM			
ACF prevalence, n (%)	524 (74.3)	167 (84.8)	<0.01
ACF number (mean ± SD)	4.1±5.0	5.3±5.7	0.04
Medication use			
NSAIDs			
ACF prevalence, n (%)	661 (76.6)	30 (76.9)	>0.99
ACF number (mean ± SD)	4.4±5.1	4.4±6.2	0.94
Aspirin			
ACF prevalence, n (%)	650 (76.9)	41 (71.9)	0.42
ACF number (mean ± SD)	4.4±5.1	4.7±5.4	0.65
Statins			
ACF prevalence, n (%)	610 (77.3)	81 (71.7)	0.19
ACF number (mean ± SD)	4.3±5.0	4.9±6.1	0.27
Metformin			
ACF prevalence, n (%)	673 (76.5)	18 (81.8)	0.80
ACF number (mean ± SD)	4.4±5.2	4.3±3.3	0.93
EPA			
ACF prevalence, n (%)	685 (76.5)	6 (85.7)	>0.99
ACF number (mean ± SD)	4.4±5.1	3.6±3.0	0.69
PPARγ agonists			
ACF prevalence, n (%)	677 (76.4)	14 (87.5)	0.39
ACF number (mean ± SD)	4.3±5.1	3.6±2.4	0.58
Insulin			
ACF prevalence, n (%)	646 (75.5%)	45 (97.8)	<0.01
ACF number (mean ± SD)	4.3±5.1	6.4±6.2	<0.01

Differences in the prevalence and number of ACF were calculated by Fisher's exact test and Student's t-test, respectively. Variable definitions: Alcohol intake was defined as positive if the subject's alcohol consumption exceeded 20 g/day. Smoking history was defined as positive if the subject had smoked >10-pack years and was still smoking or had quit within the previous 10 years. History of medication use was defined as positive if the patient had been taking the medication for at least 6 months prior to the counting of the rectal ACF. ACF, aberrant crypt foci; DM, diabetes mellitus; NSAIDs, non-steroidal anti-inflammatory drugs; EPA, eicosapentaenoic acid; PPARγ, peroxisome proliferator-activated receptor γ; SD, standard deviation.

Table III. Univariate and multivariate logistic regression analyses to identify the factors associated with the increased prevalence of ACF.

	OR (95% CI)			
Variable	Univariate	P-value	Multivariate	P-value
Older age >70 years	9.68 (5.17-18.1)	<0.01	9.19 (4.78-17.7)	<0.01
Male gender	1.63 (1.19-2.23)	<0.01	0.71 (0.54-1.03)	0.08
BMI ≥25	1.09 (0.75-1.57)	0.66		
Smoking habit	2.58 (1.85-3.61)	<0.01	1.80 (1.21-2.67)	<0.01
Alcohol intake	1.63 (1.19-2.23)	<0.01	1.18 (0.80-1.74)	0.40
Presence of colonic tumors				
Normal subject	Reference			
Adenoma cases	2.65 (1.90-3.71)	<0.01	2.40 (1.67-3.45)	<0.01
CRC cases	4.12 (2.13-7.99)	<0.01	3.15 (1.58-6.28)	<0.01
Comorbid medical conditions				
Hypertension	1.23 (0.88-1.71)	0.22		
Hyperlipidemia	1.08 (0.73-1.60)	0.70		
DM	1.92 (1.26-2.94)	<0.01	1.21 (0.75-1.96)	0.43
Medication use				
NSAIDs	1.02 (0.48-2.18)	0.96		
Aspirin	0.77 (0.42-1.40)	0.39		
Statins	0.74 (0.46-1.16)	0.19		
Metformin	1.38 (0.46-4.14)	0.56		
EPA	1.84 (0.22-15.4)	0.57		
PPARγ agonists	2.16 (0.49-9.59)	0.31		
Insulin	14.6 (2.00-106.8)	<0.01	10.2 (1.31-79.0)	0.03

Multivariate logistic regression analysis was performed with adjustment for the presence of colonic tumors. Variable definitions: Alcohol intake was defined as positive if the subject's alcohol consumption exceeded 20 g/day. Smoking history was defined as positive if the subject had smoked >10-pack years and was still smoking or had quit within the previous 10 years. History of medication use was defined as positive if the patient had been taking the medication for at least 6 months prior to the counting of the rectal ACF. ACF, aberrant crypt foci; OR, odds ratio; CI, confidence interval; CRC, colorectal cancer; DM, diabetes mellitus; NSAIDs, non-steroidal anti-inflammatory drugs; EPA, eicosapentaenoic acid; PPARγ, peroxisome proliferator-activated receptor γ; SD, standard deviation.

Table IV. Comparison of the presence of ACF between diabetic patients receiving/not receiving therapy.

Factor	Insulin therapy (+)	Insulin therapy (-)	P-value
Number of patients	46	151	
Age, years (mean ± SD)[a]	64.1±10.6	66.8±10.4	0.10
Gender (M/F)[b]	23/23	117/34	<0.01
ACF			
Prevalence, n (%)[b]	45 (97.8%)	121 (80.1%)	<0.01
Number, median (range)[c]	4.5 (2.0-7.0)	3.0 (0-6.0)	0.03

[a]P-value calculated by Student's t-test. [b]P-value calculated by the χ^2 test. [c]P-value calculated by the Mann-Whitney U test. ACF, aberrant crypt foci; SD, standard deviation; M, male; F, female.

higher than that in the patients not receiving insulin therapy (64.1 vs. 48.6%; P=0.01).

Discussion

The current study presents the results of a large, cross-sectional study conducted to investigate the associations between factors associated with the development of CRC and the presence of rectal ACF. The main aim of the study was to evaluate the potential usefulness of ACF as a surrogate biomarker of CRC, and to discuss candidate chemopreventive agents.

Consistent with previous studies (15,16,18), the present study confirmed that the prevalence and number of ACF increased significantly from normal subjects to CRC cases.

In addition, it was also confirmed that an older age, the male gender and a positive smoking habit were associated with an increased prevalence and number of ACF, as found in previous studies (12,18,23,28). The close link between tobacco smoking and an increased risk of CRC is considered to be mediated by the large number of carcinogens that can cause irreversible genetic damage to the normal colonic mucosa (29). Moreover, Stevens *et al* reported that the number of ACF was higher in patients with a family history of CRC compared with that in patients without a family history of CRC (30). In a recent study, we demonstrated that the number of ACF can be a useful predictor of the likelihood of colorectal adenoma recurrence (31). Taken together, the present results and those of previous studies lend support to the notion that ACF can serve as a reliable surrogate biomarker of CRC.

In the present study, no association could be identified between candidate chemoprevention agents and a low prevalence and number of ACF, despite these drugs having been demonstrated to prevent colorectal tumors in experimental models and/or epidemiological studies. Although this can be explained in part by the small number of patients in the subgroups of the study, particularly with regard to medication use, these results raise doubt about the validity of using ACF as an intermediate endpoint in CRC chemoprevention trials. Recently, several early-phase CRC chemoprevention trials have been conducted using ACF as an intermediate endpoint (5,6,32,33). Takayama *et al* conducted an ACF-based open-label chemoprevention trial and demonstrated the chemopreventive efficacy of sulindac (6). By contrast, a study by Limburg *et al*, in which an ACF-based randomized phase II chemoprevention trial was conducted, did not confirm the chemopreventive efficacy of sulindac (32). The differences in the prevalence of dysplastic ACF, presenting as histologically-confirmed dysplasia, may have an effect on these results. In addition, the differences in the criteria for the endoscopic identification of ACF and/or the counting area may also affect these results. Nonetheless, further investigations are required prior to incorporating these lesions into chemoprevention trials as intermediate endpoints.

Recently, a series of studies and meta-analyses confirmed that the risk for CRC is elevated in diabetic patients (26,34,35). Investigation of the molecular mechanisms for this connection has led to the so-called hyperinsulinemia hypothesis stating that insulin may, at high serum concentrations, increase the risk of CRC by acting as a mitogen and promoting the formation of colonic tumors (36). The present results indicated that insulin enhances colonic epithelial proliferation and initiates the formation of ACF, irrespective of the presence/absence of DM, emphasizing the importance of insulin in colorectal carcinogenesis. Shpitz *et al* reported that the proliferative activity of ACF was significantly increased compared with that of normal mucosa (17). Notably, at least one ACF could be identified in >60% of even normal subjects. Therefore, we hypothesized that the majority of ACF only reflect the increased proliferative activity of the colonic mucosa and that only a small proportion of ACF, which show dysplastic change, could be regarded as preneoplastic lesions of CRC. Although several studies have reported a high incidence of *KRAS* mutation in human ACF (37,38), the precise molecular trigger for the development of dysplasia in the ACF remains unknown. Therefore, additional studies are required to reveal the molecular basis and role of ACF in colorectal carcinogenesis.

The present study had certain limitations. Firstly, it was a retrospective study and patients without sufficient clinical data, including data on the behavioral characteristics, medication history and comorbid medical conditions, were excluded, therefore, a selection bias was inevitable. Secondly, the number of patients taking specific candidate chemopreventive drugs (e.g., metformin, EPA and PPARγ) was not sufficiently large, which could limit any conclusions. Thirdly, the results lacked follow-up data, and the study could not evaluate whether these clinical factors truly affected the presence of ACF. Finally, the presence of dysplastic ACF was not evaluated, as no association between the shape of the crypt lumen and the histological presence of dysplasia has been reported (39). The reported prevalence of dysplastic ACF in patients with CRC varies widely from 0.5 to 22% (10,16-18,31,40). This indicates that a consistent, reproducible set of criteria to identify dysplastic ACF by their descriptive features or a consistent scheme for their histological classification is still lacking. The histological criteria for the identification of dysplastic ACF also require standardization.

In conclusion, data from the present retrospective, large, cross-sectional study revealed the potential usefulness of ACF as a surrogate biomarker of CRC, however, useful data on candidate chemopreventive agents could not be obtained. The results indicated that insulin can enhance colonic epithelial proliferative activity and induce the formation of ACF, thereby possibly triggering CRC development.

References

1. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2013. CA Cancer J Clin 63: 11-30, 2013.
2. Das D, Arber N and Jankowski JA: Chemoprevention of colorectal cancer. Digestion 76: 51-67, 2007.
3. Niitsu Y, Takayama T, Miyanishi K, Nobuoka A, Hayashi T, Kukitsu T, Takanashi K, Ishiwatari H, Abe T, Kogawa T, *et al*: Chemoprevention of colorectal cancer. Cancer Chemother Pharmacol 54 (Suppl 1): S40-S43, 2004.
4. Boghossian S and Hawash A: Chemoprevention in colorectal cancer-where we stand and what we have learned from twenty year's experience. Surgeon 10: 43-52, 2012.
5. Hosono K, Endo H, Takahashi H, Sugiyama M, Sakai E, Uchiyama T, Suzuki K, Iida H, Sakamoto Y, Yoneda K, *et al*: Metformin suppresses colorectal aberrant crypt foci in a short-term clinical trial. Cancer Prev Res (Phila) 3: 1077-1083, 2010.
6. Takayama T, Nagashima H, Maeda M, Nojiri S, Hirayama M, Nakano Y, Takahashi Y, Sato Y, Sekikawa H, Mori M, *et al*: Randomized double-blind trial of sulindac and etodolac to eradicate aberrant crypt foci and to prevent sporadic colorectal polyps. Clin Cancer Res 17: 3803-3811, 2011.
7. Arber N, Eagle CJ, Spicak J, Rácz I, Dite P, Hajer J, Zavoral M, Lechuga MJ, Gerletti P, Tang J, *et al*: Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med 355: 885-895, 2006.
8. Grady WM and Carethers JM: Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology 135: 1079-1099, 2008.
9. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM and Bos JL: Genetic alterations during colorectal-tumor development. N Engl J Med 319: 525-532, 1988.
10. Adler DG, Gostout CJ, Sorbi D, Burgart LJ, Wang L and Harmsen WS: Endoscopic identification and quantification of aberrant crypt foci in the human colon. Gastrointest Endosc 56: 657-662, 2002.

11. Bird RP: Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: Preliminary findings. Cancer Lett 37: 147-151, 1987.
12. Moxon D, Raza M, Kenney R, Ewing R, Arozullah A, Mason JB and Carroll RE: Relationship of aging and tobacco use with the development of aberrant crypt foci in a predominantly African-American population. Clin Gastroenterol Hepatol 3: 271-278, 2005.
13. Ohkubo H, Takahashi H, Yamada E, Sakai E, Higurashi T, Uchiyama T, Hosono K, Endo H, Taguri M and Nakajima A: Natural history of human aberrant crypt foci and correlation with risk factors for colorectal cancer. Oncol Rep 27: 1475-1480, 2012.
14. Pretlow TP, Barrow BJ, Ashton WS, O'Riordan MA, Pretlow TG, Jurcisek JA and Stellato TA: Aberrant crypts: Putative preneoplastic foci in human colonic mucosa. Cancer Res 51: 1564-1567, 1991.
15. Sakai E, Takahashi H, Kato S, Uchiyama T, Hosono K, Endo H, Maeda S, Yoneda M, Taguri M and Nakajima A: Investigation of the prevalence and number of aberrant crypt foci associated with human colorectal neoplasm. Cancer Epidemiol Biomarkers Prev 20: 1918-1924, 2011.
16. Seike K, Koda K, Oda K, Kosugi C, Shimizu K, Nishimura M, Shioiri M, Takano S, Ishikura H and Miyazaki M: Assessment of rectal aberrant crypt foci by standard chromoscopy and its predictive value for colonic advanced neoplasms. Am J Gastroenterol 101: 1362-1369, 2006.
17. Shpitz B, Bomstein Y, Mekori Y, Cohen R, Kaufman Z, Neufeld D, Galkin M and Bernheim J: Aberrant crypt foci in human colons: Distribution and histomorphologic characteristics. Hum Pathol 29: 469-475, 1998.
18. Takayama T, Katsuki S, Takahashi Y, Ohi M, Nojiri S, Sakamaki S, Kato J, Kogawa K, Miyake H and Niitsu Y: Aberrant crypt foci of the colon as precursors of adenoma and cancer. N Engl J Med 339: 1277-1284, 1998.
19. Endo H, Hosono K, Fujisawa T, Takahashi H, Sugiyama M, Yoneda K, Nozaki Y, Fujita K, Yoneda M, Inamori M, *et al*: Involvement of JNK pathway in the promotion of the early stage of colorectal carcinogenesis under high-fat dietary conditions. Gut 58: 1637-1643, 2009.
20. Endo H, Hosono K, Uchiyama T, Sakai E, Sugiyama M, Takahashi H, Nakajima N, Wada K, Takeda K, Nakagama H and Nakajima A: Leptin acts as a growth factor for colorectal tumours at stages subsequent to tumour initiation in murine colon carcinogenesis. Gut 60: 1363-1371, 2011
21. McLellan E and Bird RP: Effect of disulfiram on 1,2-dimethylhydrazine- and azoxymethane-induced aberrant crypt foci. Carcinogenesis 12: 969-672, 1991.
22. McLellan EA and Bird RP: Specificity study to evaluate induction of aberrant crypts in murine colons. Cancer Res 48: 6183-6186, 1988.
23. McLellan EA, Medline A and Bird RP: Sequential analyses of the growth and morphological characteristics of aberrant crypt foci: Putative preneoplastic lesions. Cancer Res 51: 5270-5274, 1991.
24. Moghaddam AA, Woodward M and Huxley R: Obesity and risk of colorectal cancer: A meta-analysis of 31 studies with 70,000 events. Cancer Epidemiol Biomarkers Prev 16: 2533-2547, 2007.
25. Acott AA, Theus SA, Marchant-Miros KE and Mancino AT: Association of tobacco and alcohol use with earlier development of colorectal cancer: Should we modify screening guidelines? Am J Surg 196: 915-918, 2008.
26. Yuhara H, Steinmaus C, Cohen SE, Corley DA, Tei Y and Buffler PA: Is diabetes mellitus an independent risk factor for colon cancer and rectal cancer? Am J Gastroenterol 106: 1911-1921, 2011.
27. Higurashi T, Hosono K, Endo H, Takahashi H, Iida H, Uchiyama T, Ezuka A, Uchiyama S, Yamada E, Ohkubo H, *et al*: Eicosapentaenoic acid (EPA) efficacy for colorectal aberrant crypt foci (ACF): A double-blind randomized controlled trial. BMC Cancer 12: 413, 2012.
28. Mutch MG, Schoen RE, Fleshman JW, Takahashi H, Iida H, Uchiyama T, Ezuka A, Uchiyama S, Yamada E and Ohkubo H: A multicenter study of prevalence and risk factors for aberrant crypt foci. Clin Gastroenterol Hepatol 7: 568-574, 2009.
29. Luchtenborg M, White KK, Wilkens L, Kolonel LN and Le Marchand L: Smoking and colorectal cancer: Different effects by type of cigarettes? Cancer Epidemiol Biomarkers Prev 16: 1341-1347, 2007.
30. Stevens RG, Swede H, Heinen CD, Jablonski M, Grupka M, Ross B, Parente M, Tirnauer JS, Giardina C, Rajan TV, *et al*: Aberrant crypt foci in patients with a positive family history of sporadic colorectal cancer. Cancer Lett 248: 262-268, 2007.
31. Uchiyama T, Takahashi H, Endo H, Kato S, Sakai E, Hosono K, Yoneda M, Inamori M, Hippo Y, Nakagama H and Nakajima A: Number of aberrant crypt foci in the rectum is a useful surrogate marker of colorectal adenoma recurrence. Dig Endosc 24: 353-357, 2012.
32. Limburg PJ, Mahoney MR, Ziegler KL, Sontag SJ, Schoen RE, Benya R, Lawson MJ, Weinberg DS, Stoffel E, Chiorean M, *et al*: Randomized phase II trial of sulindac, atorvastatin and prebiotic dietary fiber for colorectal cancer chemoprevention. Cancer Prev Res 4: 259-269, 2011.
33. Cho NL, Redston M, Zauber AG, Carothers AM, Hornick J, Wilton A, Sontag S, Nishioka N, Giardiello FM, Saltzman JR, *et al*: Aberrant crypt foci in the adenoma prevention with celecoxib trial. Cancer Prev Res (phila) 1: 21-31, 2008.
34. Larsson SC, Orsini N and Wolk A: Diabetes mellitus and risk of colorectal cancer: A meta-analysis. J Natl Cancer Inst 97: 1679-1687, 2005.
35. Deng L, Gui Z, Zhao L, Wang J and Shen L: Diabetes mellitus and the incidence of colorectal cancer: An updated systematic review and meta-analysis. Dig Dis Sci 57: 1576-1585, 2012.
36. Giovannucci E: Insulin, insulin-like growth factors and colon cancer: A review of the evidence. J Nutr 131 (Suppl 11): S3109-S3120, 2001.
37. Smith AJ, Stern HS, Penner M, Hay K, Mitri A, Bapat BV and Gallinger S: Somatic APC and K-ras codon 12 mutations in aberrant crypt foci from human colons. Cancer Res 54: 5527-5530, 1994.
38. Takayama T, Ohi M, Hayashi T, Miyanishi K, Nobuoka A, Nakajima T, Satoh T, Takimoto R, Kato J, Sakamaki S and Niitsu Y: Analysis of K-ras, APC and beta-catenin in aberrant crypt foci in sporadic adenoma, cancer and familial adenomatous polyposis. Gastroenterology 121: 599-611, 2001.
39. Siu IM, Pretlow TG, Amini SB and Pretlow TP: Identification of dysplasia in human colonic aberrant crypt foci. Am J Pathol 150: 1805-1813, 1997.
40. Hurlstone DP and Cross SS: Role of aberrant crypt foci detected using high-magnification-chromoscopic colonoscopy in human colorectal carcinogenesis. J Gastroenterol Hepatol 20: 173-181, 2005.