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ORIGINAL ARTICLE

Aberrant crypt focus and fragile histidine triad protein in sporadic colorectal carcinoma

Kim Vaiphei, Aruna Rangan, Rajinder Singh

Kim Vaiphei, Aruna Rangan, Department of Histopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, Pin 160012, India
Rajinder Singh, Department of General Surgery, Post Graduate Institute of Medical Education and Research, Chandigarh, Pin 160012, India
Author contributions: Vaiphei K and Rangan A designed and carried out the study, including the gross assessment and sampling of the tissue blocks for the study, the immunohistochemistry and light microscopy assessment and analysis of the observations; Singh R contributed to collecting the clinical data and collection of the surgically resected specimens for the study; Vaiphei K and Rangan A drafted the article, revisiting the important intellect content; and final approval of the manuscript was given by all three authors.
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Correspondence to: Dr. Kim Vaiphei, Professor, Department of Histopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, Pin 160012, India. kvaiphei2009@gmail.com
Telephone: +91-172-2755140 Fax: +91-172-2744401
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Abstract

AIM: To characterize aberrant crypt focus (ACF) in adjoining mucosa in sporadic colorectal carcinoma and to evaluate fragile histidine triad (Fhit) protein and Ki67.

METHODS: ACF was identified grossly and classified histologically in 75 resected specimens. ACF was typed into hyperplastic ACF (HACF) and dysplastic ACF (DACF). Sections of ACF, carcinoma and normal colonic mucosa as control were studied for Fhit and Ki67 expressions by immunohistochemistry and were grouped according to staining intensity and the number of positive stained cells observed in different histological groups. Comparison was done between the different groups by Pearson's χ^2 test and γ test for the ordinal data. P value < 0.05 was considered as significant.

RESULTS: Age range was 40 to 86 years in males (mean = 43.36) and 45 to 70 years in females (mean = 56). HACF was identified in all cases studied in the non-tumorous colonic mucosa; ACF was observed as non-contiguous scattered foci, which supports the hypothesis of acquisition of single focus monoclonality by colonic epithelial cells in tumor generation. Twenty-four (32%) had DACF and were observed as closure to carcinoma foci. Intensity of Fhit expression: (1) HACF - 40% exhibited strong intensity, similar to normal, moderate in 36% and weak in 24%; (2) DACF - strong in 25%, moderate in 37.5% and weak in 37.5%; and (3) carcinoma - negative in 16%, strong in 43% and moderate and weak in 28.5% each. Significant difference was observed in intensity of the Fhit protein expressions by HACF and DACF (P < 0.05). Tumor in older patients showed a stronger Fhit intensity compared to younger patients (P = 0.036). Vegetarian diet intake and non-smokers showed stronger Fhit intensities. Advanced stage tumor, non-vegetarian diet and younger age was associated with loss of Fhit protein. Ki67 positivity was an extended crypt pattern in HACF and DACF showed extension up to the neck region of the crypts and surface epithelium. Carcinomas showed a marked increase in Ki67 expression (P < 0.05). Fhit protein had an inverse association with Ki67 expression.

CONCLUSION: Weaker Fhit intensity was associated with smoking, non-vegetarian diet intake and increasing Ki67 expression. Loss of Fhit protein expression is possibly influenced by environmental factors like smoking and non-vegetarian diet intake.

Key words: Aberrant crypt focus; Carcinogenesis, Colorectal carcinoma; Dysplasia; Fragile histidine triad protein; Ki67

Peer reviewer: Vittorio Ricci, MD, PhD, Associate Professor, Director, Laboratory of Cellular and Molecular Gastroenterology, Department of Physiology, Human Physiology Section,

University of Pavia Medical School, Via Forlanini 6, 27100 Pavia, Italy

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common causes of cancer-related deaths worldwide[1]. Almost half of patients present in advanced tumor stage and 5-year survival rate in patients with non-localized tumor is only 11%[1]. Therapeutic options, such as surgery, radiotherapy and chemotherapy, are mostly ineffective against advanced stage cancer. Moreover, these therapeutic agents produce high toxicity and unacceptable side effects[2-4]. Identifying early tumor in human colonic mucosa is an important step in tumor prevention and progress. The age standardized rates of CRC in India is 4.2 and 3.2/100 000 for males and females respectively[5]. Aberrant crypt focus (ACF), first described in the early 1990s by Bird *et al*[6], has been recognized as a putative pre-neoplastic lesion preceding an adenoma in the newly proposed ACF-Adenoma-Carcinoma sequence[7]. Presumably ACF is the result of crypt fission giving rise to early flat lesion. It can be classified histologically into simple, hyperplastic, dysplastic and combination of hyperplastic and dysplastic types[8,9]. The incidence of ACF varies widely and Roncucci *et al*[10] and Jass *et al*[11] found at least one ACF in each sample from colorectal carcinoma. One recent publication reported a 20% increase risk (non significant) for CRC in current smokers with high fruit and vegetable consumption[12]. The billion dollar Women's Health Initiative randomized trial also failed to find a reduction in CRC in participants randomized to the low fat, high fruit and vegetable arm[13]. A report from the Kashmir valley dominated by a population with a non-vegetarian diet reported a similar incidence to other parts of the country[14]. Hence, besides a dietary factor in the carcinogenetic pathway of CRC, other aspects of personal habits and lifestyle need to be studied in more detail in the epidemiology of CRC.

The fragile histidine triad (*Fhit*) gene is a candidate tumor suppressor gene located at chromosome 3p14.2 and encompassing the FRA3B common fragile site[15]. Abnormal expressions of *Fhit* gene has been identified in various human cancers and cancer cell lines, including gastrointestinal cancers. Genetic and epigenetic alterations result in homozygous genomic deletions in Fhit gene and down regulation of the Fhit protein in various carcinomas[16-19]. In lung cancer, aberrant Fhit protein expression and inactivation of the Fhit gene has been attributed to smoking[18,20,21]. Various types of cancers, including CRC, are documented to have a strong association with dietary habits and life style[22-32]. However, isolated studies in CRC reported normal Fhit gene with no mutational changes and loss of heterozygosity. Hao *et al*[30] and Cao *et al*[33] demonstrated a gradational loss of Fhit protein expression by pre-neoplastic colorectal lesions. Ki-67, a proliferative marker, identifies the proliferating cell population topologically restricted to a lower third of a normal crypt[31]. Neoplastic colorectal epithelial cells are often reflected in loss of topological organization and acquisition of diffuse and increased Ki67 expression[32]. Sporadic colon cancer is believed to be related to epigenetic change rather than germ line mutation and largely affected by dietary factors and life style[22].

Identifying an early molecular marker helps in better understanding of the carcinogenetic pathway, thereby giving the scope for timely intervention in disease prevention. The dominant form of CRC in India is the sporadic type, contributing more than 90%, and it is the type of CRC with strong epigenetic influence. The present study was carried out in order to characterize ACF in the non-carcinomatous colonic mucosa and to analyze Fhit protein expression pattern and cell proliferative index indicated by Ki67. These parameters were correlated with clinical profiles and tumor characteristics.

MATERIALS AND METHODS

The study included 75 resected specimens of sporadic CRC. All samples were subjected to routine grossing. Adjoining mucosa was examined for ACF, identifiable as roughened or granular elevated foci with a central depressed area (Figure 1A). Additional tissue samplings were taken from these areas for histological characterization and immunohistochemistry examination. Paraffin blocks bearing the tumor and ACF were cut at a 2 micron thickness and were used for immunohistochemistry staining by the peroxidase anti-peroxidase technique after antigen retrieval. Antigen retrieval was carried out by the pressure cooker method in a jar containing 0.01 mm/L citric acid at pH 6.0. Primary antibodies used in the study were anti-Fhit protein (monoclonal, 1:100 dilution; Zymed Laboratories, California) and Ki67 (monoclonal, 1:50 dilution; Dako, Denmark). A similar number of endoscopic biopsy of colon and rectum showing normal histology were used as normal control. Envision detection system (Dako, Denmark) was used. Histologically, ACF was classified into simple, hyperplastic, dysplastic and mixed types. For each case, the clinical history was analyzed and tumors were described with respect to site, histological type, TNM staging, lymph node status, dietary habits and smoking.

Ethical consideration

The study was approved by the Ethics Committee of the Post Graduate Institute of Medical Education and Research, Chandigarh, India. Informed consent was taken from all the patients enrolled in the study after explaining utilization of the tissue material for the study and the possible outcome with the clinical implication. The

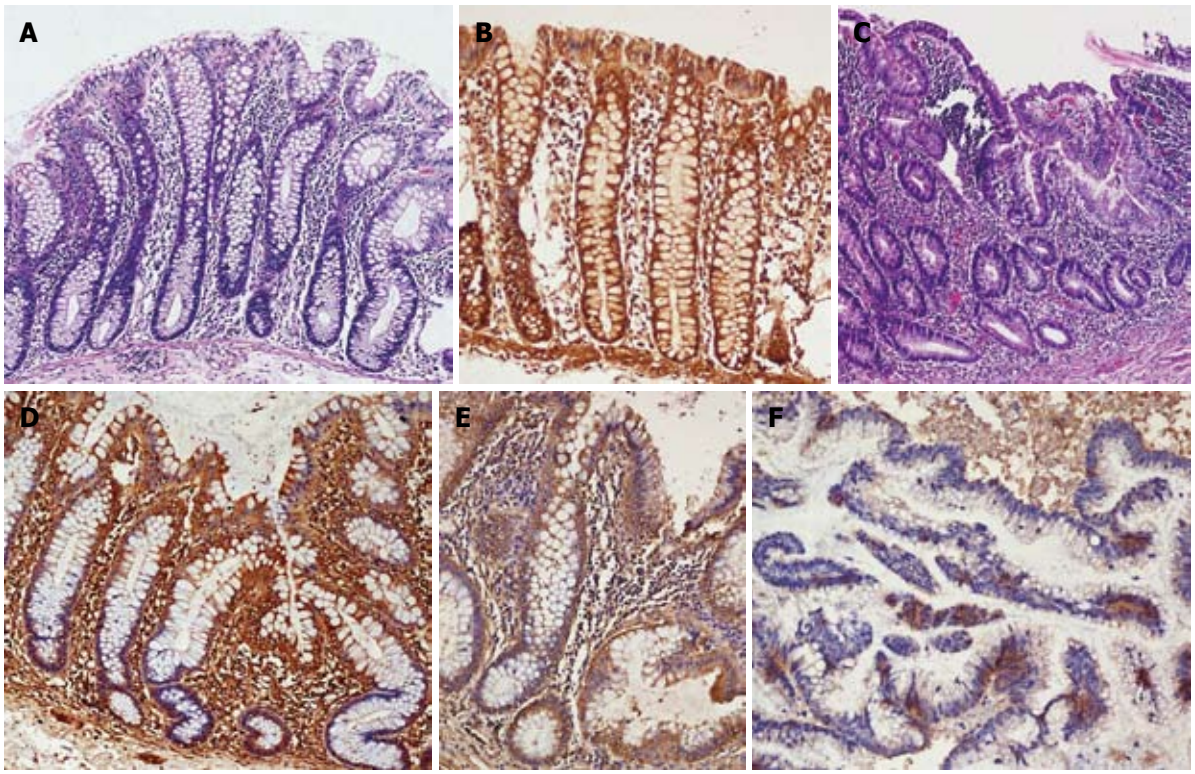

Figure 1 Medium power photomicrograph. A: Medium power photomicrograph of colonic mucosa taken from the areas of granular mucosa showing hyperplastic aberrant crypt focus (ACF) [hematoxylin eosin (HE), × 300]; B: Medium power photomicrograph of normal colonic mucosa showing diffuse and strong expression for Fhit protein [peroxidase anti-peroxidase (PAP), × 300]; C: Medium power photomicrograph of colonic mucosa taken from the granular mucosa closed to the tumor showing dysplastic ACF (HE, × 250); D: Medium power photomicrograph of hyperplastic ACF showing diffuse but weaker cytoplasmic Fhit protein expression (PAP, × 300); E: Medium power photomicrograph of dysplastic ACF showing weak cytoplasmic Fhit protein expression (PAP, × 250); F: Medium power photomicrograph of a colonic adenocarcinoma showing a negative staining for Fhit protein (PAP, × 250).

patients were also made aware that their confidentiality would be maintained and no additional invasive procedure from the routine surgical procedure would be required for the study. They could withdraw from the study at any given time, which would not change the patient management and clinician relationship.

Assessing Fhit protein and Ki67 expressions and Fhit gene

Normal colonic epithelium showed a positive staining. Immunohistochemistry stainings were put up side by side for normal control, ACF and carcinoma sections. The intensity of staining for Fhit protein was scored as negative, weak, moderate and strong, as in normal mucosa. As for Ki67, basal lower 1/3rd crypt nuclear positivity was taken as normal and classified as mid third, neck region and surface epithelial cell positivity.

Statistical analysis

Staining intensity of Fhit protein in different histological types of ACF and tumor tissue were compared and correlated with Ki67 positivity and clinical parameters and tumor variables. Nominal data such as sex, diet, smoking history and tumor details, including tumor metastasis, were analyzed using Pearson's χ^2 test. Ordinal data such as age, tumor differentiation, infiltration into wall of the gut and nodal involvement were analyzed by the γ test. Tests were considered significant when $P < 0.05$.

RESULTS

Out of 75 cases studied, 57 (76%) were male with a sex ratio of M:F = 4:1. Age range was 40 to 86 years in males (mean = 43.36 years) and between 45 to 70 years in females (mean = 56 years), 54 (72%) patients were older than 50 years of age. 57 (76%) patients were non-vegetarian, consuming a mixed diet of chicken and fish 3 to 4 times per week, and 27 (36%) patients were smokers. Tumor size: 42 (56%) tumors were less than 5 cm, 30 (40%) were between 5-10 cm and 3 (4%) were more than 10 cm in diameter. Tumors were present in the ascending colon in 36 (48%), transverse colon in 9 (12%), descending colon in 6 (8%) and rectum in 24 (32%) patients. Well differentiated tumor was observed in 9 (12%), moderately differentiated in 54 (72%) and poorly differentiated in 12 (16%) patients. The tumors were classified as T2 in 3 (4%), T3 in 63 (84%) and T4 in 9 (12%) and T4 had infiltration into the adjacent ileal loop. Nodal metastasis was observed in 18 cases, N1 in 15 (20%) and N2 in 3 (4%), and 3 (4%) patients also had metastasis to the liver. TNM staging: stage Ⅰ = 3, stage ⅡA = 30, stage ⅡB = 24, stage ⅢB = 12, stage ⅢC = 3 and stage Ⅳ = 3 cases. Three (4%) cases had mild inflammation, 39 (52%) had moderate inflammation, and 24 (32%) had heavy inflammation along with lymphoid aggregates seen in 12 (16%) cases, 6 cases each with moderate to dense aggregates.

Table 1 Percentage of Fhit protein expression in hyperplastic aberrant crypt focus, dysplastic aberrant crypt focus and carcinoma *n* (%)

Fhit intensity	HACF	DACF	Carcinoma
No staining	0	0	12 (16)
Weak	18 (24)	9 (37.5)	18 (24)
Moderate	27 (36)	9 (37.5)	18 (24)
Strong	30 (40)	6 (25)	27 (36)
Total cases	75 (100)	24 (100)	75 (100)

HACF: Hyperplastic aberrant crypt focus; DACF: Dysplastic aberrant crypt focus; Fhit: Fragile histidine triad.

Table 2 Comparison of Fhit intensity between hyperplastic aberrant crypt focus, dysplastic aberrant crypt focus and carcinoma

Paired difference	mean	SD	*t* differentiation	*P* value
HACF *vs* DACF	0.25	0.46	1.528	0.17
HACF *vs* carcinoma	0.0952	0.77	0.568	0.576

HACF: Hyperplastic aberrant crypt focus; DACF: Dysplastic aberrant crypt focus.

Desmoplasia was mild in 24 (32%) cases, moderate in 36 (48%) and marked in 15 (20%) cases.

ACF

Grossly, many ACF were identified adjacent to the tumor. Prominent and larger foci were identified closer to the tumor. Histology showed hyperplastic ACF (HACF, Figure 1A) in 100% of the cases. In HACF, the crypts appeared broad, longer lined by tall columnar epithelium and increased number of goblet cells. The crypt lumen in these foci appeared with an irregular lumen compared to a normal crypt which tends to have round or oval uniform lumen. Each ACF contained numerous crypts. ACF with low grade dysplasia (LDACF, Figure 1C) was observed in 24 (32%) cases. DACF showed epithelial cells with an increased nucleo-cytoplasmic ratio, nuclear hyperchromasia and mild epithelial cell stratification. DACF on the whole were larger on gross examination and closer to the carcinoma focus. We observed more than 3 crypts per focus which were frequently confluent. ACF immediate to carcinoma focus appeared to be more in number but were discontinuous along the colonic length.

Fhit expression

Strong cytoplasmic immunostaining was observed in the normal control (Figure 1B). Other cells like fibroblasts, endothelial cells and lymphocytes also showed cytoplasmic expression for Fhit protein. Fhit positivity was graded as weak, moderate and strong (Table 1). Fhit cytoplasmic positivity in HACF was strong, as in the normal control (Figure 1D), in 30 (40%), moderate in 27 (36%) and weak in 18 (24%). Of the 24 DACF, strong immunostaining as in normal mucosa was observed in only 6 (25%), moderate in 9 (37.5%) and weak or no staining in 9 (37.5%) (Figure 1E). Among the carcinomas, 12 (16%) were negative (Figure 1F), strong cytoplasmic expression was observed in 27 (36%), moderate and weak in 18 (24%) each. Mean numbers of patients with different intensities in each group were compared (Table 2). A higher number of HACF showed stronger intensity than DACF and DACF more than carcinoma; the paired differences, however, were not significant ($P > 0.05$). Gender did not have any bearing on Fhit expressions in HACF, DACF or in carcinoma (Table 3). Fhit expression was variable in different groups. Tumors in patients older than 50 years of age showed more cases with strong intensity than those less than 50 years ($P = 0.036$). HACF showed strong intensity irrespective of the age of patients, DACF showed stronger Fhit intensity in patients who were older than 50 years of age (Table 4). Of personal habits, 57 (76%) were non-vegetarian and 18 (24%) were vegetarian. HACF and carcinoma in non-vegetarians had more patients with weak or moderate intensity compared to vegetarians (Table 5) but the difference did not yield any significant difference. Smoking was observed in 27 (36%) patients and 48 (64%) were non-smokers. Strong intensity of Fhit staining was exhibited by non-smokers compared to smokers ($P < 0.001$), with no differences between weak and moderate intensities. Smoking did not show any significant influence in Fhit expression by carcinoma cells ($P > 0.05$) (Table 6).

Tumor distribution: 36 (48%) tumors were present in the ascending colon, 9 (12%) in the transverse colon, 6 (8%) in the descending colon and 24 (32%) in the rectum. The intensities and Fhit positivity did not have any association with tumor site. Distribution of histological grading in the 63 tumors which were Fhit positive was 3 (5%) well differentiated, 51 (81%) moderately differentiated and 9 (14%) poorly differentiated. There were no significant differences in the intensity of Fhit staining with the tumor gradings (Table 7). TNM staging of the tumors did not show any difference in the Fhit expression (Table 8), although intensity of the Fhit protein in tumors invading adjacent organs was significantly weaker or negative compared to those tumors which had invaded only up to the serosa ($P < 0.05$). Regional lymph node metastasis did not have any influence in the Fhit expression in ACF and tumors.

Ki67 expression in ACF in relationship to Fhit protein

Ki67 expression showed crypt oriented patterns with normal colonic mucosa showing a basal pattern. HACF showed expanded bases and upper third positivity. DACF showed surface epithelium positivity. Crypt oriented Ki67 positive pattern in relationship to intensity of Fhit protein expressions is shown in Table 9. More HACF showed a stronger intensity of Fhit protein i.e., 40% of the cases showed +++ Fhit expression than DACF, where only 25% (6/24) cases showed +++ positivity, with significant difference ($P < 0.05$). DAFC cases not only showed higher number of Ki67 positivity, but also showed an expanded pattern of Ki67 positivity extending up to the neck region of the crypts and along the surface

Table 3 Fhit in hyperplastic aberrant crypt focus, dysplastic aberrant crypt focus and carcinoma *vs* sex

Fhit intensity	Hyperplastic ACF (P = 0.521)			Dysplastic ACF (P = 0.915)			Carcinoma (P = 0.269)		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Weak	12	6	18	6	3	9	18		18
Moderate	24	3	27	6	3	9	15	3	18
Strong	21	9	30	3	3	6	18	9	27
Total	57	18	75	15	9	24	51	12	63

Fhit: Fragile histidine triad; ACF: Aberrant crypt focus.

Table 4 Fhit in hyperplastic aberrant crypt focus, dysplastic aberrant crypt focus and carcinoma *vs* age

Fhit intensity	HACF (P = 0.265)			DACF (P = 0.655)			Carcinoma (P = 0.036)		
	≤ 50 yr	> 50 yr	Total	≤ 50 yr	> 50 yr	Total	≤ 50 yr	> 50 yr	Total
Weak	9	9	18		9	9	6	12	18
Moderate	6	21	27	6	3	9	9	9	18
Strong	6	24	30		6	6		27	27
Total	21	54	75	6	18	24	15	48	63

Fhit: Fragile histidine triad; HACF: Hyperplastic aberrant crypt focus; DACF: Dysplastic aberrant crypt focus.

Table 5 Fhit in hyperplastic aberrant crypt focus, dysplastic aberrant crypt focus and carcinoma *vs* vegetarian and non-vegetarian

Fhit intensity	HACF (P = 0.035)			DACF (P = 0.076)			Carcinoma (P = 0.037)		
	Veg	Non Veg	Total	Veg	Non Veg	Total	Veg	Non Veg	Total
Weak	3	15	18	3	6	9		18	18
Moderate		27	27		9	9		18	18
Strong	15	15	30	6		6	12	15	27
Total	18	57	75	9	15	24	12	51	63

Fhit: Fragile histidine triad; Veg: Vegetarian; HACF: Hyperplastic aberrant crypt focus; DACF: Dysplastic aberrant crypt focus.

Table 6 Fhit intensity in hyperplastic aberrant crypt focus, dysplastic aberrant crypt focus and carcinoma *vs* smoking

Fhit intensity	HACF (P = 0.007)			DACF (P = 0.641)			Carcinoma (P = 0.213)		
	Non Smoker	Smoker	Total	Non Smoker	Smoker	Total	Non Smoker	Smoker	Total
Weak	9	9	18	6	3	9	12	6	18
Moderate	9	18	27	6	3	9	6	12	18
Strong	30		30	6		6	21	6	27
Total	48	27	75	18	6	24	39	24	63

Fhit: Fragile histidine triad; HACF: Hyperplastic aberrant crypt focus; DACF: Dysplastic aberrant crypt focus.

Table 7 Fhit intensity in hyperplastic aberrant crypt focus, dysplastic aberrant crypt focus and carcinoma *vs* tumor differentiation

Tumor grade	Hyperplastic ACF (P = 0.719)				Dysplastic ACF (P = 0.232)				Carcinoma (P = 0.444)				
Fhit→	+	+ +	+ + +	Total	+	+ +	+ + +	Total	-ive	+	+ +	+ + +	Total
Well diff		3		3	3			3	7	3			10
Mod diff	15	18	27	60	6	9	6	21	3	9	15	27	54
Poorly diff	3	6	3	12					2	6	3		11
Total	18	27	30	75	9	9	6	24	12	18	18	27	75

Fragile histidine triad (Fhit) protein grading: + = weak; ++ = moderate; +++ = strong; diff = differentiation; mod = moderate. ACF: Aberrant crypt focus.

epithelium, whereas HACF showed basal or expanded basal patterns with no surface epithelium staining. Ki67 in the carcinoma group had all tumor cells showing positive staining, irrespective of the Fhit protein staining pattern which was dominantly weak to moderate intensity.

Other tumor variables were compared with Fhit stain-

Table 8 Fhit intensity in aberrant crypt focus *vs* tumor-node-metastasis stage

Fhit intensity	T (P = 0.325)					N (P = 0.938)			M (P = 0.396)		
	Tis	T1	T2	T3	T4	N0	N1	N2	M0	M1	Total
Negative				9	3	12			12		12
Weak			3	14	1	10	8		18		18
Moderate				16	2	8	7	3	15	3	18
Strong				24	3	27	0		27		27
Total			3	63	9	57	15	3	72	3	75

Fhit: Fragile histidine triad; T: Tumor size; N: Lymph nodes; M: Metastasis.

Table 9 Ki67 expression pattern and Fhit intensity inhyperplastic aberrant crypt focus, dysplastic aberrant crypt focus

Ki67	HACF (P = 0.617)				DACF (P = 0.243)			
Fhit intensity→	+	+ +	+ + +	Total	+	+ +	+ + +	Total
Basal			8	8				
Expanded basal	5	9	13	27				
Neck	13	18	9	40	3	2	5	10
Surface					6	7	1	14
Total	18	27	30	75	9	9	6	24

Fragile histidine triad (Fhit) intensity: + = weak; ++ = mod (moderate); +++ = strong. HACF: Hyperplastic aberrant crypt focus; DACF: Dysplastic aberrant crypt focus.

ing using the t-test and γ test, depending upon whether the data was nominal or ordinal. Variables, such as site (P = 0.345), tumor grade (P = 0.719), tumor type (P = 0.0681), tumor inflammatory cell infiltration (P = 0.757), lymphoid aggregation (P = 0.768) and desmoplasia (P = 0.061), did not influence the intensity of Fhit protein expression in HACF. Transmural tumor infiltration with serosal involvement (P = 0.325), nodal involvement (P = 0.938) and tumor metastasis (P = 0.396) did not show any significant association. Extension of tumor infiltration into the adjacent organ resulted in significantly reduced Fhit protein staining intensity (P = 0.029).

To summarize, we observed significant correlation between Fhit protein expression and clinical parameters like age, smoking and dietary habits. Patients who were older than 50 years of age showed a stronger intensity for Fhit protein by tumor cells than those tumors from patients who were younger than 50 years of age (P = 0.036). A significant reduction in Fhit protein expression by HACF was seen among smokers (P = 0.007) and non-vegetarians (P = 0.035), and non-smokers and non-vegetarians (P = 0.037). HACF from patients with larger tumor size (i.e., > 5 cm) appeared to show a stronger intensity than those HACF from patients with tumor < 5 cm in size (P = 0.017). Significantly weaker Fhit protein expression was observed in those tumors that had invaded adjacent organs (P = 0.029). Fhit protein intensity was inversely related with Ki67 expression among the ACF and DACF with surface epithelial staining for Ki67 showed significantly lesser number of cases with +++ Fhit intensity. A gradually decreasing intensity of Fhit protein in the normal control, HACF, DACF and carcinoma were associated with a gradually increasing Ki67 expression.

DISCUSSION

We observed HACF in all the resected specimens enrolled in the present study, thereby indicating the presence of ACF in the human colon to be a common phenomenon. We also documented DACF to be closer to the tumor foci, supporting the new concept of ACF to be the earliest precursor lesion in the pathogenetic pathway of colorectal carcinogenesis[34-36]. Recognition of DACF close to carcinoma foci was also observed in a few previous studies[10,37]. The role of ACF in CRC development has also been extensively studied and supported in experimental animal models[38-41]. Histologically, we observed more than 3 crypts per focus of aberrant crypts which were frequently confluent. ACF immediate to the carcinoma focus appeared more in number but were discontinuous along the colonic length and were diffuse, expansile and complex by branching, similar to the observation made by Roncucci *et al*[10] where they reported 0.4 ACF per square cm in adjacent colonic mucosa of colorectal carcinoma and at least one ACF in every case. However, they could not demonstrate any difference in frequency of ACF in the immediate adjacent areas within 5 cm and areas remote from the tumor. Furthermore, our observations of the focal and patchy nature of ACF observed in our cases support the hypothesis that colon cancer tends to originate as a focal genetic event in the cells of an individual crypt which exhibit monoclonality[42]. The affected crypt thereby undergoes fission to give rise to an early flat lesion like ACF[6,38].

Similarly to our observations, gradual reductions in the intensity of Fhit protein had been reported by other groups in colon cancer and its early precursor lesions, and also in other pre-neoplastic lesions in breast, pan-

creas, cervix, lung and esophagus[15-18,20,21,25-31,33,43,44]. Functional loss of the tumor suppressor protein Fhit, like p53, is believed to result in loss of inhibitory function in cell proliferation due to release phenomena in many downstream proteins acting on cell proliferations[16-19,45]. More recently, down regulation of Fhit protein expression has been associated with DNA fragility and damage[46], and also a close link between the location of the fragile gene with many of the known breakpoints in cancer specific chromosomal translocations[47].

Fhit staining intensity in our patients had a strong association with smoking and non-vegetarian dietary intakes, thereby re-confirming the previous hypothesis of epigenetic factors in deletions and dysregulation in *Fhit* gene, as in lung cancer development[16-19,21,22,24,48]. Many of our carcinoma cases were negative for Fhit protein although expression for the protein was retained by a couple of cases which could be the result of a dysregulated gene that have been observed in other tumors, including lungs and breast[20,21,24,33,43,48]. Our observation in the normal control was similar to Hao *et al*[30] where they documented cytoplasmic expression in normal colonic epithelial cells from basal onwards to luminal differentiated cells. We noted a significant influence of reduced intensity for Fhit protein in tissue from those patients who have an intake of non-vegetarian diet compared to those who had history of intake of vegetarian diet with respect to HACF ($P = 0.035$) and carcinoma ($P = 0.037$). There is only one study available in a Medline search of Fhit gene with respect to dietary factors in nasopharyngeal cancer where no loss of heterozygosity had been found[49]. We also observed that differential expressions by the Fhit protein in tumors in patients who were older than 50 years of age had minimal or no reduction compared to patients who were younger than 50 years of age with significant difference ($P = 0.036$). A study in gastric carcinoma showed a similar observation in tumor tissue between patients who were older than 50 years of age and those patients who were younger than 50 years of age[24]. Some studies also had reported frequent loss of Fhit protein expression by non-colonic tumors in the older age group, associated with poor tumor outcome[33,50]. We observed a significantly weaker or negative Fhit in tumors invading adjacent organs compared to tumors invading up to the serosa ($P = 0.029$). Previous studies have reported that higher histological grade tumors and advanced tumor stage are more likely to be negative for Fhit protein[30,33,50].

To conclude, we observed a gradual reduction in the intensity of Fhit protein expressions by HACF, DACF and carcinomas, thereby confirming loss of the tumor suppressor property by the *Fhit* gene in sporadic colorectal carcinoma tumor genesis. Our findings also showed a strong association between loss of the Fhit protein with intake of a non-vegetarian diet and smoking, the known factors for down regulation of the *Fhit* gene in the carcinogenesis pathways and reported in other types of cancers. We also observed gradual reduction in Fhit protein expression associated with increased cell proliferative index indicated by Ki67 immunostaining, thereby suggesting a release phenomenon and/or loss of control over increasing cell proliferation from normal mucosa *via* pre-cursor lesion (ACF) to cancer, in the development of CRC.

COMMENTS

Background

In spite of the gross advancement in post operative treatment modality, prognosis in colorectal cancer has not improved due to late detection of the cancer. Hence, the present study is an attempt to identify the changes that might occur at the molecular level in pre-neoplastic colonic epithelium by studying the fragile histidine triad (Fhit) protein expression by different types of colonic mucosa, including the carcinoma cells.

Research frontiers

The *Fhit* gene is a candidate tumor suppressor gene and abnormal expression has been identified in various human cancers and in cancer cell lines. Genetic and epigenetic alterations result in homozygous genomic deletions in Fhit gene and down regulation of the Fhit protein in various carcinomas and few studies demonstrated gradational loss of Fhit protein expression by pre-neoplastic colorectal lesions. Ki-67, a proliferative marker, identifies the proliferating cell population topologically.

Innovations and breakthroughs

Most of the studies reporting mutation in *Fhit* genes were related to smoking. Effect on the Fhit protein by environmental factors in sporadic colorectal carcinogenetic pathway had not been analyzed before. Similar studies had been carried out in hereditary form of colon cancer.

Applications

By understanding the role of epigenetic factors like smoking in the colorectal carcinoma (CRC) pathway, preventive measures may be implicated. By observing the expression of the Fhit protein by non-cancerous colonic epithelium, timely intervention may help to prevent development of frank carcinoma.

Terminology

Aberrant crypt focus (ACF) is terminology used for a focal or localized abnormal feature exhibited by colorectal mucosa, observed as depressed areas by the naked eye as the result of increased size of the colonic crypt lumen. Histologically, they may look similar to normal mucosa or may show hyperplasia or dysplastic epithelium. Fhit and Ki67 are proteins involved in cell cycle regulation. Fhit is a tumor suppressor protein, whereas Ki-67 is indicator of cell proliferation.

Peer review

The authors examined the expression of Fhit protein and Ki-67 in different colorectal mucosa sampled from non carcinomatous areas in sporadic CRC patients. Fhit protein showed a gradational reduction in normal mucosa, hyperplastic and dysplastic ACF and carcinomas associated inversely with Ki-67 expression. The results are interesting and may represent a molecular mechanism of esophageal carcinogenesis.

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