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RAPID COMMUNICATION

Loss of fragile histidine triad protein expression in inflammatory bowel disease

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Abstract

AIM: To investigate the expression of fragile histidine triad (FHIT) protein in 64 patients with ulcerative colitis (UC) and Crohn's disease (CD), and its relation with clinicopathological data.

METHODS: Rabbit-anti-FHIT antibody was used to detect FHIT protein expression in 64 formalin-fixed, paraffin-embedded tissue specimens of inflammatory bowel disease (IBD) by citrate-microwave-streptavidin (SP)-HRP immunohistochemical method.

RESULTS: The positive FHIT protein expression was 22.79% ± 16.16%, 42.14% ± 16.82% in active and remittent phases of UC, 36.07% ± 19.23% in CD, and 57.05% ± 8.86% in normal colon mucosa. Statistically significant differences in FHIT protein expression were observed between the active and remittent phases of UC, between the active phase of UC and normal colon mucosa, as well as between the remittent phase of UC and normal colon mucosa, and between CD and normal colon mucosa.

CONCLUSION: Our results show that FHIT protein expression is completely absent or reduced in IBD, suggesting that the FHIT gene might be associated with the oncogenesis and progression of IBD, an early event from inflammatory conditions to carcinoma in IBD.

Key words: Fragile histidine triad protein expression; Ulcerative colitis; Crohn's disease; Inflammatory bowel disease

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INTRODUCTION

Inflammatory bowel disease (IBD) is a collection of chronic idiopathic inflammatory disorders of the intestine and/or colon, including two independent diseases: ulcerative colitis (UC) and Crohn's disease (CD)[1]. Up to now, the complex etiology and pathogenesis of IBD are not known with certainty, but the growing theories suggest that many factors such as environment, genetic alteration, uncontrolled immune system, *etc*, can result in chronic gut inflammation[2-7]. However, none of the theories provides a sufficient explanation of either disease, indicating that their etiology is multifactorial. It has been well established that colorectal carcinoma is the most serious complication of patients with long-standing IBD who have an increased risk of developing colorectal carcinoma[8-11]. The cumulative risk of carcinoma in IBD patients is estimated to be 10-20 times greater in the small bowel and 4-20 times greater in the large bowel than that in the small and large bowel of general population. The mean duration of colitis before cancer diagnosis ranges 17-20 years. Dysplasia occurring in IBD constitutes a precursor stage of carcinoma[12,13]. Patients with IBD are characterized by recurrent acute mucosa inflammation, mucosal ulceration, epithelial necrosis and regeneration, all of which may result in DNA damage, genetic alterations including enhanced microsatellite instability of mucosa, activation of oncogene, inactivation of tumor suppressor gene, increasing susceptibility to mutagenesis and subsequent neoplastic transformation[8-18].

The fragile histidine triad (FHIT) gene was discovered at human chromosome 3p14.2 in 1996 by Ohta *et al*[19] using the exon trapping method, and has been identified as a candidate tumor-suppressor gene. This gene not only spans the translocation breakpoint of familial renal-cell carcinoma, but also encompasses the most active common human chromosomal fragile region, FRA3B[19,20]. The approximately 1-megabase FHIT gene includes 10 exons, encoding 1.1Kb mRNA transcript, 16.8 kDa, and 147 amino acid proteins[21]. FHIT protein is a member of the recently discovered histidine triad (HIT) family of nucleotide-binding proteins with a high specific hydrolysing activity for diadenosine 5', 5'''-P1, Pn-polyphosphate (ApnA), where n = 3-6. The FHIT protein encoded by the FHIT gene can hydrolyze AP3A

and AP4A to ADP and AMP[20,22]. It was reported that the FHIT gene can facilitate deletions and aberrant transcripts and may play an important role in a variety of human malignancies[23-26], including cancers of lung[27], breast[28], pancreas[29], urinary bladder[30], head and neck[31] and gastrointestinal carcinomas[32-34].

Since the role of FHIT aberrations is unclear and results from different investigators are contradictory, the present study was to evaluate the expression of FHIT protein in patients with IBD and its relation with clinicopathological data.

MATERIALS AND METHODS

Patients

Biopsy specimens from 64 IBD patients including 47 UC and 17 CD patients were obtained from several hospitals in Hubei Province from 1990 to 2005. All the patients were diagnosed in the light of clinical, endoscopic, histological and radiological criteria. All IBD patients underwent sigmoidoscopy or colonscopy for routine clinical evaluation. Mucosal inflammation in active UC was classified as mild inflammation: mild-to-moderate small round-cell infiltration with formation of a few crypt abscesses in the lamina propria; severe inflammation: severe small round-cell infiltration with multiple crypt abscesses and partial granulation in the lamina propria. Remission was defined histologically as areas with branched or regenerated irregular crypts without acute inflammation but with chronic mild inflammation. Ten apparently normal colonic tissue sections were obtained. The mean age of 47 patients including 34 men and 13 women at the onset of UC was 36.9 years (averaged 13.4 years). The mean age of the remaining 17 patients including 10 men and 7 women at the onset of CD was 38.0 years (averaged 13.9 years).

Reagents and antibodies

Rabbit anti-FHIT polyclonal antibody (Zymed Company, USA) was purchased from Beijing Zhongshan Biological Technology CO, Ltd. Immunohistochemical staining S-P kit (Zemed MAXIM, USA) was purchased from Maixin Co, LTD.

Methods

All the biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin wax. Four-μM thick sections were routinely prepared and stained with haematoxylin and eosin, while other sections were prepared with immunohistochemical SP staining method. In brief, the tissue sections were routinely dewaxed in xylene, rehydrated at graded concentrations of alcohol, and treated with 3% hydrogen peroxide for 10 min to block the endogenous peroxidase. The sections were immersed in citrate buffer (5 mmol/L sodium citrate, pH = 6.0), heated in a microwave oven for 2 min to enhance antigen retrieval, and then placed at room temperature for 10 min. The sections were blocked with normal goat serum for 15 min and incubated with rabbit anti-FHIT polyclonal antibody (1:100 dilution) overnight at 4℃. After washed three times in PBS, the binding of antibodies to their antigenic sites in the tissue sections was further amplified with biotinylated goat anti-rabbit antibody followed by reaction with streptavidin-biotin peroxidase. Antibody localization was detected with diaminobenzidine as a chromogen substrate. Sections prepared by substituting PBS for the primary antibody served as a negative control. The freshly prepared substrate DAB was added for color development. The sections were washed in distilled water and counterstained with haematoxylin and mounted for examination.

Determination of FHIT expression

Negatively expressed FHIT manifested as blue-stained nuclei while positively expressed FHIT manifested as brown or dark brown cytoplasm and/or cell membrane mainly in epithelial tissues. Expressions of these target proteins were semi-quantitated with automatic image analyzer (Nikon,Japan) and HPIAS-2000 image analyzing program, in which the average value of positive cells in 10 randomly selected high power fields (× 400) for each section was used for the comparison of the target protein expressions.

Statistical analysis

Quantitative variables were expressed as mean ± SD. Statistical comparisons between groups were made by one-way ANOVA. Differences in results between the two groups were tested with t test. $P < 0.05$ was considered statistically significant. All analyses were performed using the SPSS version 14.0.

RESULTS

The classification of IBD patients according to their clinicopathological characteristics and their association with FHIT protein expression are shown in Tables 1 and 2.

The pattern of FHIT protein expression was confined to the epithelial cells, especially cytoplasm. As shown in Figure 1A-1D, FHIT protein expression was unequivocal in normal colonic tissue and reduced or absent in UC and CD. The positive FHIT expression was 22.79% ± 16.16%, 42.14% ± 16.82%, respectively in active and remittent phases of UC, 36.07% ± 19.23% in CD, and 57.05% ± 8.86% in normal colon mucosa. Statistically significant differences in FHIT protein expression were observed between the active and remittent phases of UC ($P < 0.05$), between the active phase of UC and normal colon mucosa ($P < 0.01$), as well as, between the remittent phase of UC and normal colon mucosa ($P < 0.01$), and between CD and normal colon mucosa ($P < 0.01$).

DISCUSSION

Recent studies showed that tumourigenesis is related to inflammatory conditions, such as IBD and pancreatitis, and inflammation has been considered as precancerosis, but the possible link between inflammation and tumourigenes is still unclear[14,35,36]. Chronic inflammatory conditions such as UC are thought as the risk factor for some carcinomas, the incidence of colon carcinoma in UC is estimated to be 5-7 times greater than what we have expected,

Table 1 Clinicopathological data of patients with inflammatory bowel disease

Patient No.	Sex	Age (yr)	Disease extension
Crohn's disease			
1	M	54	Terminal ileum
2	F	20	Ileocolonic
3	M	40	Pancolitis
4	M	70	Right-sided
5	F	62	Ileocolonic
6	M	27	Terminal ileum
7	M	26	Terminal ileum
8	F	34	Ileocolonic
9	M	51	Pancolitis
10	M	41	Pancolitis
11	M	29	Right-sided
12	F	25	Left-sided
13	M	35	Terminal ileum
14	F	38	Ileocolonic
15	F	27	Terminal ileum
16	M	31	Right-sided
17	M	42	Ileocolonic
Ulcerative colitis-active			
18	M	37	Ileocolonic
19	M	45	Rectosigmoid
20	M	42	Rectosigmoid
21	M	58	Rectosigmoid
22	M	28	Rectosigmoid
23	F	26	Rectosigmoid
24	M	36	Rectosigmoid
25	M	27	Rectosigmoid
26	M	32	Rectosigmoid
27	F	47	Rectosigmoid
28	M	33	Rectosigmoid
29	M	33	Rectosigmoid
30	M	36	Left-sided
31	F	44	Rectosigmoid
32	M	41	Rectosigmoid
33	M	24	Left-sided
34	F	58	Rectosigmoid
35	F	50	Rectosigmoid
36	M	64	Rectosigmoid
37	M	48	Rectosigmoid
38	M	60	Pancolitis
39	M	35	Pancolitis
40	M	30	Rectosigmoid
41	M	13	Left-sided
42	M	52	Pancolitis
43	F	38	Pancolitis
44	M	17	Pancolitis
45	F	26	Left-sided
46	M	37	Pancolitis
47	M	20	Rectosigmoid
48	M	43	Pancolitis
49	F	51	Pancolitis
50	M	32	Rectosigmoid
Ulcerative colitis-Remittent			
51	M	8	Ileocolonic
52	M	18	Rectosigmoid
53	F	35	Rectosigmoid
54	F	24	Rectosigmoid
55	M	36	Rectosigmoid
56	F	35	Rectosigmoid
57	M	35	Pancolitis
58	F	41	Pancolitis
59	M	13	Rectosigmoid
60	F	44	Pancolitis
61	F	45	Pancolitis
62	M	60	Rectosigmoid
63	M	23	Rectosigmoid
64	M	56	Left-sided

Table 2 Comparison of FHIT protein immunohistochemical expression in ulcerative colitis, Crohn's disease, and normal colon mucosa (mean ± SD)

Groups	*n*	Positive rate of FHIT protein expression (%)
Normal colon mucosa	10	57.05 ± 8.86
Ulcerative colitis		
Active	33	22.79 ± 16.16
Remittent	14	42.14 ± 16.82
Crohn's disease	17	36.07 ± 19.23

and colon carcinoma occurs in 20%-35% patients with IBD. Compared with sporadic colorectal cancers, such as adenomatous polyposis and hereditary non-polyposis colorectal cancer syndrome, the prognosis of UC-associated colorectal cancer is the worst, the 5-year survival rate of patients is the lowest (< 40%)[10,12]. In our study, the positive rate of FHIT expression was 31.88% ± 20.33%, 22.79% ± 16.16%, 42.14% ± 16.82% in initial, active and remittent phases of UC, and 36.07% ± 19.23% in CD, and 57.05% ± 8.86% in normal colon mucosa. Statistically significant differences in FHIT protein expression were observed between the active and remittent phases of UC, between the active phase of UC and normal colon mucosa, as well as between the remittent phase of UC and normal colon mucosa, and between CD and normal colon mucosa. The severer the inflammation (active phase) is, the more reduction the FHIT protein expression is.

The possible interpretations for these results are as follows. IBD is strongly related to environment, and microsatellite instability in 50% UC patients is enhanced during inflammation. Microsatellites are simple repetitive sequences of DNA that are scattered throughout the genome. These sequences are stably inherited, varying from individual to individual, and have a low alteration rate. Instability within these sequences has been recognized as a marker for genome mutations and DNA repair deficiency. Recently, there have been some reports on detecting microsatellite instability in non-neoplastic settings, including inflammatory mucosa of IBD[8]. Inflammation results in an increase in DNA damage, strengthening the ability of cells to repair the damage before replication[37]. Mutations of oncogene and tumor suppressor gene may be the initiating events in tumourigenesis arising from an inflammatory backgroud, and continuous production of mutations may be required for tumor progression[38]. Patients with IBD are characterized by recurrent acute inflammation of the mucosa, mucosal ulceration, and epithelial necrosis and regeneration. Various kinetic analyses have shown increasing epithelial cell proliferation or cell death in crypts and dysplastic glands in IBD, especially in UC[9,36]. Active inflammation and regeneration may increase epithelial cell turnover, susceptibility to mutagenesis, and neoplastic transformation[39,40]. The positive rate of FHIT expression in remission of UC was 42.14% ± 16.82%. The possible theoretical explanation is that the abnormal architecture after active ulceration might impair mucosal function and lead to increased cell turnover. Another possibility is that remission in UC is

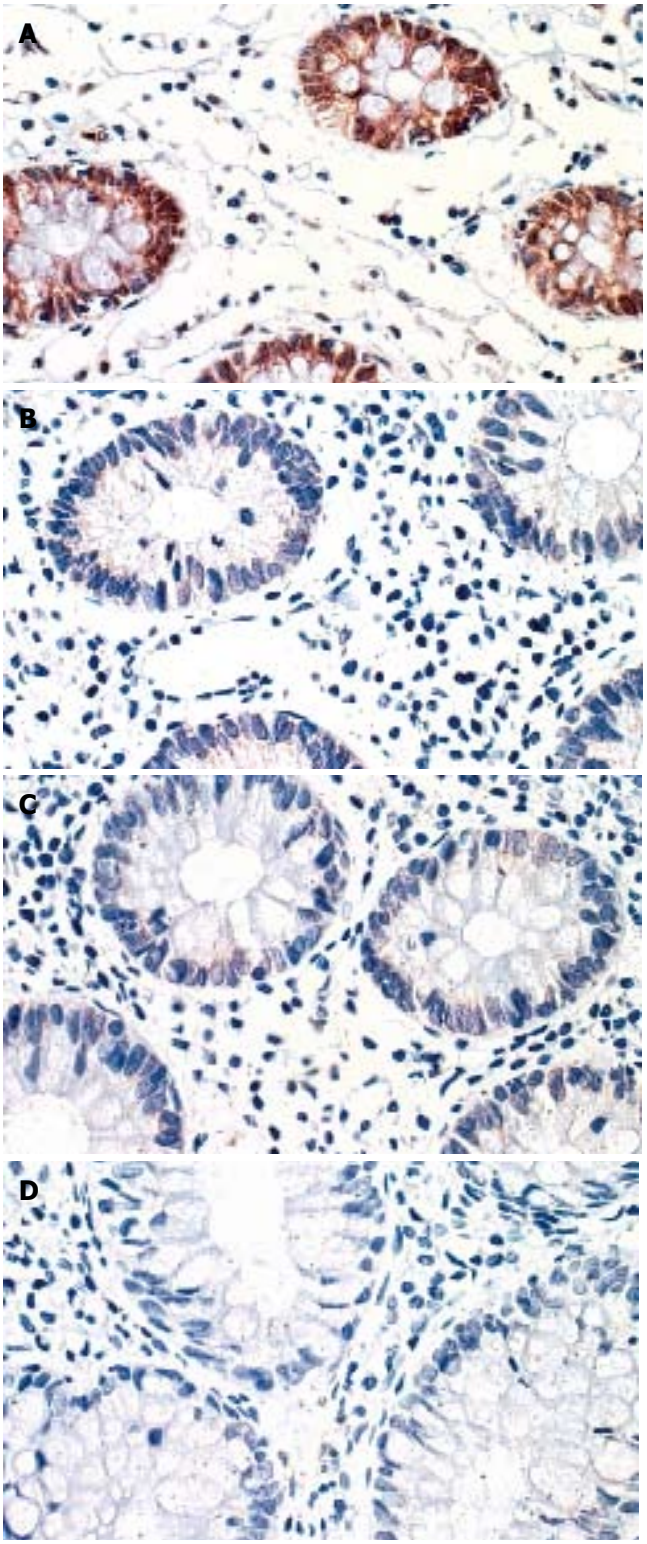

Figure 1 FHIT protein expression in normal colonic epithelium (**A**), ulcerative colitis (**B**, **C**), and Crohn's disease (**D**) (SP × 400).

only relative with mucosal damage at a subclinical and subhistologic level between the time points of obvious relapse.

Chronic inflammation and epithelial cell damage that characterize IBD result in increased cell proliferation and cell death[41]. At the same time, the accelerated cell turnover prediposes to genetic alterations in mucosa, which is in line with dysplasia and carcinoma in IBD. Nobuyasu Arai *et al*[9] suggested that P53 accumulation and high P21WAF1/CIP1 expression accelerate epithelial cell turnover and may result in an elevated risk of developing dysplasia and carcinoma in patients with IBD.

Chronic inflammation and epithelial damage might predispose to DNA damage in mucosa and accelerate the gene mutation. In the present study, FHIT-protein immunohistochemical expression was absent or reduced in IBD, which might be a precursor from inflammation to carcinoma transformation, suggesting that the alteration of FHIT-protein expression might remain long before any histological morphological change[10]. Skopelitou AS *et al*[42] showed that FHIT protein immunostaining is completely absent or reduced in most *H pylori*-related chronic gastritis, which may play a role in the development and progression of gastric cancer. The reduced or absent FHIT protein expression during inflammation is due to the direct exposure of the gastrointestinal tract to environmental factors that induce FHIT/FRA3B breakage and sustain FHIT damage as an early event or due to DNA damage and FHIT gene mutation induced by chronic inflammation[43].

Reduced or absent FHIT protein expression may play a role in accelerating epithelial cell turnover or carcinoma transformation in IBD. Diadenosine triphosphase is first sequestered and eventually hydrolyzed by FHIT to ADP and AMP, the balance between cellular AP3A level and FHIT enzymatic activity may affect cell death or survival. Reduced or absent FHIT protein expression can inhibit the enzymatic activity of FHIT and increase the cellular AP3A level.AP3A can strengthen the transmission of cellular survival signals and accelerate the epithelial cell turnover. It has been suggested that AP4A can also be hydrolyzed by FHIT to ADP and AMP. AP4A is an intracellular regulatory molecule which may regulate the ability of cells to adapt to metabolic stresses such as oxidation and DNA damage. When the function of FHIT protein is diminished, the normal level of AP4A is deviated, which may result in the inability of cells to adapt to environmental stresses and cause genetic damage. At the same time, the ratio of AP3A to AP4A is increased, which can also increase cellular survival[10,20-23,28,30,32-34]. FHIT protein is involved in the regulation of apoptosis in cell culture systems, which is independent of P53, Bax or Bcl-2 expression and has been considered as an independent mechanism for tumor suppression. FHIT-induced apoptosis comprises signaling processes of FHIT-caspase 8-caspase 9-Bid-PARP, which has two pathways: one is activated by caspase-8, the other involves bypassing mitochondria[31,32]. When FHIT protein is abnormal, apoptosis is disrupted and cellular proliferation is accelerated. FHIT is involved in the regulation of cell cycle and DNA retrieval, FHIT protein can transform damaged DNA into S phase[44]. When FHIT protein expression is reduced or absent, disruption of cell cycle regulation leads to uncontrolled proliferation and formation of tumors.

In conclusion, the high frequency of complete absence and/or reduced immunohistochemical expression of FHIT protein suggests that the FHIT gene might be involved in

most cases of IBD, which might be a precursor from the inflammatory conditions to carcinoma transformation.

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