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• *BRIEF REPORTS* •

Clinicopathological significance of FHIT protein expression in gastric adenocarcinoma patients

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Abstract

AIM: To investigate the expression of fragile histidine triad (FHIT) protein, and the possible relationship between FHIT expression and clinicopathological indices in gastric carcinoma.

METHODS: FHIT protein expression was examined in 76 cases of gastric carcinoma, 58 cases of intraepithelial neoplasia, and 76 cases of corresponding normal mucosae by immunohistochemical method to analyze its relationship to histological grade, clinical stage, metastatic status and prognosis.

RESULTS: The FHIT protein expression was positive in 28/76 (36.8%) cases of adenocarcinoma tissue, 22/58 (37.9%) cases of adjacent dysplastic tissue and 76/76 (100%) cases of distal normal gastric mucosa. There was a significant difference in the expression of FHIT protein between cancer or adjacent intraepithelial neoplasia and normal gastric mucosa ($P = 0.000$). FHIT protein expression was found in 64.3% (18/28) of grades I and II cancers, and 20.8% (10/48) of grade III cancers ($P = 0.000$), in 56.3% (18/32) of stages I and II cancers and 22.7% (10/44) of stages III and IV cancers ($P = 0.004$), and in 63.6% (14/22) of cancers without metastasis but only 25.9% (14/54) of those with metastasis ($P = 0.003$). The significant difference in the expression of FHIT was found between histological grade, clinical stage and metastatic status of cancer. Follow-up data showed that there was a significant difference in median survival time between cancer patients with expression of FHIT (71 mo) and those without (33 mo, log rank = 20.78, $P = 0.000$).

CONCLUSION: FHIT protein is an important tumor suppressor protein. Loss of FHIT protein expression may be associated with carcinogenesis, invasion, metastasis and prognosis of gastric adenocarcinoma.

Key words: Gastric cancer; Gene expression; FHIT; Prognosis

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INTRODUCTION

Fragile histidine triad (FHIT) gene, localized on chromosome 3p14.2 is an important tumor suppressor gene identified after Rb, p53, and p16 genes[1,2]. It spans not only the *t* (3:8) (p14.2; q24) translocation breakpoint found in familial renal cell carcinoma but also the most common human fragile site, FRA3B[2,3]. Alterations in the FHIT gene and/or its expression have been found in primary tumors and cell lines of lung[4,5], breast[6-8], head and neck[9], esophagus[10-12], stomach[13-15], colon and rectum[16-18], pancreas[19], kidney[20,21], cervix[22-25], and hepatocellular carcinoma[26-30]. Abnormal protein expression and allelic deletion of FHIT in lung cancer are associated with the history of smoking and prognosis[31,32]. The finding of decreased expression of FHIT in 93% of precancerous lesions of the lung suggests that this gene might be used as an intermediate biomarker for early diagnosis and/or prevention of lung cancers[32]. Gastric cancer, like lung cancer, is thought to be induced by carcinogens such as *Helicobacter pylori*, alcohol, smoking, high salt and nitrosamine, and low antioxidant vitamins and is increasing in frequency among males in many countries. Therefore, it is imperative to determine whether FHIT plays a role in this second-ranked tumor in male cancer mortality, which has been increasing in China since the 1990s. Only a few reports have evaluated the FHIT gene in primary gastric carcinoma so far[13-15,34-36]. Photomicrographs of FHIT protein expression in gastric carcinoma have been reported[34-36]. However, there is no detailed investigation of FHIT protein expression during gastric carcinogenesis in Chinese patients. Based on the study carried out in 76 gastric adenocarcinomas with 58 abutting intraepithelial neoplasia tissues and 76 distal normal gastric mucosae, the FHIT protein expression in Chinese patients is altered in a high proportion of gastric carcinomas as well as adjacent intraepithelial neoplasia and the loss of FHIT expression is significantly correlated with more advanced clinical stage, poorer differentiation, metastasis and worse prognosis of gastric cancer.

MATERIALS AND METHODS

Biopsy specimens

Paraffin-embedded sections of 76 gastric carcinomas with corresponding 58 adjacent intraepithelial neoplasia mucosae

and 76 distal normal gastric tissues were obtained from the Department of Pathology, Chinese People's Liberation Army General Hospital (Beijing, China). The age of patients ranged from 35 to 84 years, averaged 59±11.58 years. Sixty-six were men and 10 women. Among them, 22 had grade I carcinoma, 6 had grade II carcinoma and 48 had grade III carcinoma, according to their histological grading; while 20 had stage I carcinoma, 12 had stage II carcinoma, 38 had stage III and 6 had stage IV carcinoma, according to the clinical staging of TNM, respectively. Lymphatic metastasis in regional nodes was confirmed during surgery in 54 cancers.

Immunohistochemistry

All samples were fixed in 10% buffered formalin and embedded in paraffin. Four-micrometer-thick sections were cut from wax blocks, mounted onto APES-coated glass slides. Slides were deparaffinized twice in xylene for 10 min, rehydrated through graded ethanol to distilled water before incubation for 30 min with 3% hydrogen peroxidase-methanol to inhibit endogenous peroxidase activity, and heated in 0.01 mol/L citrate buffer (pH 6.0) in a microwave oven for 5 min at 100 ℃ for antigen retrieval. Then the slides were taken out of the microwave oven to be cooled at room temperature for 30 min. After being incubated for 15 min in a blocking solution containing 10% normal goat serum in PBS, sections were incubated at 4 ℃ overnight in a humidified chamber with rabbit polyclonal antibody to human FHIT (Zymed Laboratories Inc., South San Francisco, CA, USA) diluted 1:200 in blocking solution. The sections were rinsed in PBS and incubated for 30 min with biotinylated secondary antibody (Histostain-SP, Zymed). After being washed in PBS, the sections were then incubated for 30 min in streptavidin–HRP (Histostain-SP, Zymed). 3,3'-Diaminobenzidine was used as the chromogen. Slides were counterstained for 3 min with hematoxylin solution. Normal liver tissue was used as a positive control for each lesion, whereas the primary antibody was replaced by normal rabbit serum IgG at a similar dilution or PBS as a negative control.

Evaluation of score

In scoring FHIT protein expression, both the extent and intensity of immunopositivity were considered, according to Hao *et al*.[18]. The intensity of positivity was scored as follows: 0, negative; 1, weak; 2, moderate; 3, strong as the normal stomach. The extent of positivity was scored as follows: 0, <5; 1, >5-25%; 2, >25-50%; 3, >50-75%; 4, >75% of the cells in the respective lesions. The final score was determined by multiplying the intensity of positivity and the extent of positivity scores, yielding a range from 0 to 12. Scores 9-12 were defined as preserved or strong staining pattern (++), 5-8 as weak staining pattern (+), and 0-4 were markedly reduced or negative expression (-).

Statistical analysis

Fisher's exact test (two-sided), Pearson's χ^2 test for trends in proportions and Kaplan-Meier method with log rank test for survival analysis were used to assess the associations between FHIT expression and pathological indices by SPSS 10.0 for Windows (Chicago, IL, USA). $P<0.05$ was considered statistically significant.

RESULTS

FHIT expression in normal, adjacent dysplastic mucosae and adenocarcinoma

FHIT protein was predominantly strongly expressed in the cytoplasm of epithelial cells in 76/76 distal normal stomach mucosae (Figure 1A) and 22/58 (37.9%) adjacent intraepithelial neoplasia. Some stromal cells, such as fibroblasts, endoepithelial cells, and macrophages, also expressed FHIT protein, both in nuclei and cytoplasm. FHIT protein was positively expressed in 28/76 (36.8%) of gastric adenocarcinomas. The carcinomas with markedly reduced or loss of FHIT protein were 48/76 (63.2%), in which the extent and intensity of FHIT expression were markedly reduced or absent in cancer cells (Figure 1B). A significant difference was found in the expression of FHIT protein between normal gastric mucosae and adenocarcinoma or adjacent intraepithelial neoplasia ($P = 0.000$). There was no difference in FHIT expression between carcinoma and adjacent intraepithelial neoplasia ($P = 1.000$).

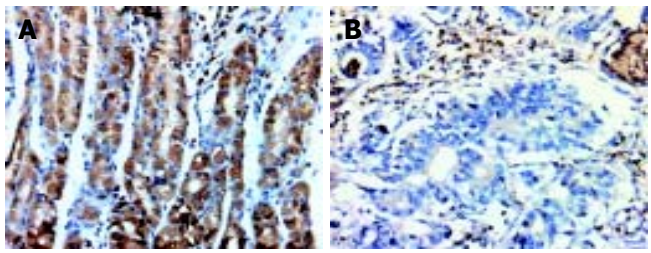

Figure 1 Positive FHIT expression in the normal gastric mucosa (**A**) and negative FHIT expression in cancer cells (**B**). (SP ×200).

Relationship between FHIT expression and histological grade, clinical stage and prognosis

The proportion of carcinomas with expression of FHIT protein showed a decreasing trend from 18 of 28 (64.3%) well- and moderately-differentiated cancers (grades I and II) to 10 of 48 (20.8%) poorly-differentiated carcinomas (grade III), and a significant inverse association was found between FHIT expression and histological grade ($P = 0.000$). A decreasing trend in FHIT expression was also observed in clinical stage, from 18 of 32 (56.3%) in stages I and II adenocarcinomas to 10 of 44 (22.7%) in stages III and IV adenocarcinomas and there was a significant difference in FHIT expression between clinical stages ($P = 0.004$) as well. FHIT expression was present in 14 of 54 (25.9%) cancers with metastasis or in 14 of 22 (63.6%) of tumors without metastasis, thus a significant difference in expression of FHIT was found between the adenocarcinomas with different biological behaviors ($P = 0.003$). Follow-up data showed that there was a significant difference in median survival time between the carcinoma patients with FHIT expression (71 mo) and those without FHIT expression (33 mo, log rank = 20.78, $P = 0.000$, Figure 2).

DISCUSSION

Although FHIT protein is expressed in most types of normal human tissues, it is frequently reduced or lost in a variety

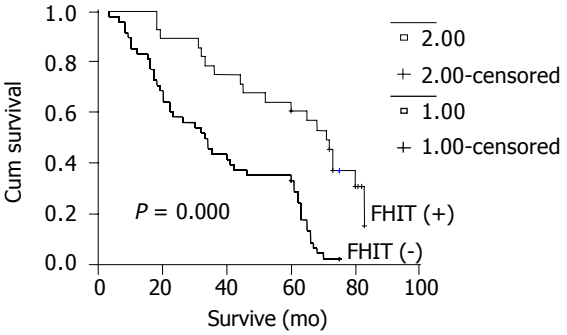

Figure 2 Difference in median survival time between carcinoma patients with and without FHIT expression (n = 76). The y-axis represents the percentage of patients, the x-axis represents their survival time. The red line represents FHIT-positive patients with a better survival, the green line represents FHIT-negative gastric carcinoma patients (log rank = 20.78; P = 0.000).

of human tumors due to alterations in its gene transcription or gene deletion[1-3]. It has thus been suggested that FHIT gene is a candidate tumor suppressor gene for multiple carcinomas. FHIT gene protein is a member of histidine triad family and the mechanism of its suppression on tumor cells remains obscure[1-3]. The following possible mechanisms have been considered as a tumor suppressor[33]. First, the tumor-suppressing function of FHIT might catabolize ApppA (Ap_3A) or related substrates. Ap_3A is an analog of ATP, which can provide phosphates as a substrate to raise the activity of protein kinase. Loss of FHIT protein may lead to the loss of Ap_3A hydrolase activity and the resulting elevated levels of Ap_3A or similar compounds may enhance the transductive signals of growth, thus contributing to carcinogenesis. Second, the activity of FHIT on mRNA cap analogs raises the possibility that failure of a decapping function might be tumorigenic, but the properties of FHIT are quite different from those of enzymes known to decap mRNA, making this an unlikely mechanism. Third, the tumor-suppressing function of FHIT might be the signaling of FHIT-substrate complexes or compounds as an active form of FHIT. Fourth, FHIT might have a nucleotide-independent role as a tumor suppressor[33]. The loss of FHIT protein corresponds to the carcinogenesis and evolution of gastric adenocarcinoma. Capuzzi *et al*.[34], found that 49% of tumors show complete loss plus 29% partial loss of expression in FHIT protein. The absence of FHIT protein is related to higher histological grade and higher tumor stage. Lee *et al*.[35], observed that FHIT expression correlates with the prognosis of gastric cancer. Rocco *et al*.[36], showed that loss of FHIT protein expression is associated with progression and poor differentiation of gastric cancer. Our results are consistent with those reported outside China[34-36], and our previous finding in Chinese colorectal cancer and hepatocellular carcinoma patients[17]. Besides adenocarcinoma, we have also investigated the adjacent intraepithelial neoplasia mucosa and found only 37.9% of which could express FHIT protein, slightly higher than that of carcinoma (36.8%). Therefore, it is also further suggested that like lung and esophageal cancers associated with environmental carcinogens, loss of FHIT protein plays an important role in gastric cancer transformation at its early stage and thus the treatment of FHIT in molecular approach can prevent the carcinogenesis of precancerous gastric lesions.

In conclusion, FHIT protein, like Rb, p53 and p16, may be a universal tumor-suppressor protein, and plays an important role in the carcinogenesis, progress and prognosis of Chinese gastric carcinoma patients. The FHIT expression status detected by immunohistochemistry may be used as a simple and useful molecular marker for the prognosis of gastric adenocarcinoma patients.

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