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• LIVER CANCER •

Loss of fragile histidine triad protein in human hepatocellular carcinoma

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Abstract

AIM: To investigate the expression of fragile histidine triad (FHIT) gene protein, Fhit, which is recently thought to be a candidate tumor suppressor. Abnormal expression of fragile histidine triad has been found in a variety of human cancers, but little is known about its expression in human hepatocellular carcinogenesis and evolution.

METHODS: Sections of 83 primary human hepatocellular carcionoma with corresponding para-neoplastic liver tissue and 10 normal liver tissue were evaluated immunohistochemically for Fhit protein expression.

RESULTS: All normal liver tissue and para-neoplastic liver tissue showed a strong expression of Fhit, whereas 50 of 83 (65.0 %) carcinomas showed a marked loss or absence of Fhit expression. The differences of Fhit expression between carcinoma and normal or para-neoplastic liver tissue were highly significant (P=0.000). The proportion of carcinomas with reduced Fhit expression showed an increasing trend (a) with decreasing differentiation or higher histological grade (P=0.219); (b) in tumors with higher clinical stage III and IV (91.3 %, P=0.000), compared with tumors with lower stage I and II (27.6 %); and (c) in cancers with bigger tumor size (>50 mm) (75.0 %, P=0.017), compared with smaller tumor size ($\leqslant$ 50 mm).

CONCLUSION: FHIT inactivation seems to be both an early and a later event, associated with carcinogenesis and progression to more aggressive hepatocellular carcinomas. Thus, evaluation of Fhit expression by immunohistochemistry in hepatocellular carcinoma may provide important diagnostic and prognostic information in clinical application.

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INTRODUCTION

Fragile histidine triad gene (FHIT) has been cloned and mapped to chromosomal region 3p14.2[1]. It spans the t(3:8)+(p14.2;q24) translocation breakpoint found in familial renal cell carcinoma and encompasses the most common human fragile site, FRA3B[2,3]. Alterations of FHIT and its expression have been found in primary tumors and cell lines of the lung[4,5], breast[6-8], head and neck[9], esophageal[10-12], stomach[13-15], colon and rectum[16-18], pancreas[19], kidney[20,21], cervix[22-25], and hepatocellular carcinomas[26-30]. Allelic deletion of FHIT and abnormal expression of FHIT protein (Fhit) in lung cancer are associated with smoking history and poor prognosis[31, 32]. The finding of decreased expression of Fhit in 93 % of precancerous lesions of the lung suggests that this gene may be used as a molecular marker for early diagnosis and prognosis of lung cancers[32]. However, there are only a few reports that evaluated FHIT in hepatocellular carcinoma in a small number of cases so far[30] and further investigation of Fhit protein expression during hepatocellular carcinogenesis is required. Liver cancer, like lung cancer, is thought to be induced by carcinogens such as major viral and environmental risk factors. Therefore, it is imperative to determine whether FHIT plays a role in the development of hepatocellular carcinoma which has been ranked the second in cancer mortality in China since 1990s and is increasing in the rate of its incidence among males in many countries. In this study, 10 normal liver tissues and 83 hepatocellular carcinomas with their corresponding para-neoplastic liver tissues were examined for Fhit expression by immunohistochemistry. It was found that the expression of Fhit was altered in a high proportion of hepatocellular carcinomas and the loss of Fhit expression was associated with more advanced stage of the tumor.

MATERIALS AND METHODS

Specimens

Paraffin embedded sections of 83 hepatocellular carcinomas with corresponding para-neoplastic tissues and 10 normal liver tissues as controls were obtained from the Department of Pathology, Chinese People's Liberation Army General Hospital. The patients included 71 men and 12 women with the mean age of 52±9.67 years (range 10-76 years). Of these patients, 15 were at grade I, 39 at grade II and 29 at grade III according to histological grading; and 4 were at stage I, 33 at stage II, 46 at stage III and 4 at stage IV according to clinical staging of UICC. In terms of size, 44 tumors were bigger than and 39 were equal to or smaller than 50 mm in diameter.

Immunohistochemical determination of Fhit

All specimens were fixed in 10 % buffered formalin and embedded in paraffin. Paraffin blocks were sectioned into 4-μm thickness and the sections were mounted onto APES-coated glass slides. The slides were deparaffinized in xylene twice for 10 minutes, rehydrated through graded ethanol to distilled water, incubated for 30 minutes with 3 % hydrogen peroxidase-methanol to inhibit endogenous peroxidase activity, and heated in 0.01M citrate buffer (pH 6.0) in a microwave oven for 5 minutes at 100 ℃ for antigen retrieval. After cooled down at room temperature for 30 minutes, the slides were blocked for 15 minutes in PBS containing 10 % normal goat serum, incubated at 4 ℃ overnight in a humidified chamber with rabbit polyclonal antibody to human Fhit (Zymed Laboratories Inc., South San Francisco, CA) at 1:200 dilution in blocking solution. The sections were then rinsed in PBS and incubated

for 30 minutes with biotinylated secondary antibody (Histostain-SP, Zymed), rinsed again in PBS and incubated for 30 minutes in streptavidin-HRP (Histostain-SP, Zymed). 3, 3' -Diaminobenzidine was used as the chromogen. Slides were counterstained for 3 minutes with hematoxylin solution. Normal liver tissue was used as the positive control for each lesion, whereas the primary antibody was replaced by normal rabbit serum IgG with a similar dilution or PBS for the negative control.

Evaluation of score

Both the extent and intensity of immunostaining were considered when scoring Fhit protein expression according to Hao *et al*[18]. The intensity of positive staining was scored as 0, negative; 1, weak; 2, moderate; 3, strong as in normal liver. The extent of positive staining was scored as 0, <5; 1, >5-25 %; 2, >25-50 %; 3, >50-75 %; 4, >75 % of the hepatocytes in the respective lesions. The final score was determined by multiplying the intensity score and the extent score, yielding a range from 0 to 12. Scores 9-12 were defined as preserved or strong staining (++), 5-8 as weak staining (+) and 0-4 as markedly reduced or negative expression (-).

Statistical analysis

Fisher' s exact test (two sided) and Pearson Chi square' s test for trends in proportions were used to assess the associations between Fhit expression and pathological indices. A $P<0.05$ was considered statistically significant.

RESULTS

Fhit expression in normal, para-neoplastic tissue and carcinoma

Fhit was strongly expressed in the cytoplasm of hepatocytes in all 10 normal liver and 83 para-neoplastic tissues (Figure 1A). Some stromal cells, such as lymphocytes, plasma cells and macrophages, also expressed Fhit in both nucleus and cytoplasm. The expression of Fhit was strong in 33, weak in 21 and negative in 29 hepatocellular carcinomas (Table 1). The carcinomas with markedly reduced or loss of Fhit expression were observed in 50 (65.2 %) cases, whereas those with expression of Fhit equal to normal liver were observed in 33 (34.8 %) cases. In cases with reduced expression of Fhit, both the extent and intensity of Fhit staining were reduced markedly (Figure 1B).

Table 1 Levels of Fhit expression in heptocellular carcinomas, para-neoplastic tissues and normal liver tissues

	Fhit score			
	n	-	+	++
HCC	83	29	21	33
Para-neoplastic tissue[b]	83	0	0	83
Normal liver tissue[b]	10	0	0	10

[b]P=0.000, *vs* hepatocellular carcinomas.

Relationship between Fhit expression and histological grade, clinical stage and tumor size

The percentage of carcinomas with reduced expression of Fhit increased from 46.7 % (7 of 15) in well-differentiated cancers (grade I) to 53.8 % (21 of 39) in moderately differentiated cancers (grade II) and to 75.8 % (22 of 29) in poorly differentiated cancers (grade III), although this association of increased histological grade of tumors with decreased Fhit expression was not statistically significant (P>0.05, Table 2). Nevertheless, the decrease in expression of Fhit was significantly associated with more advanced clinical stage of the tumors. Whereas 21.6 % (8 of 37) stage I and II cases showed reduced expression of Fhit, the percentage of stage III and IV cases with reduced expression of Hhit increased to 91.3 % (42 of 46) (P=0.000, Table 2). In addition, the carcinomas with reduced expression of Fhit protein were found in 75 % (33 of 44) of tumors greater than 50 mm in diameter, compared with 43.6 % (17 of 39) of tumors 50 mm or smaller in diameter (P=0.017).

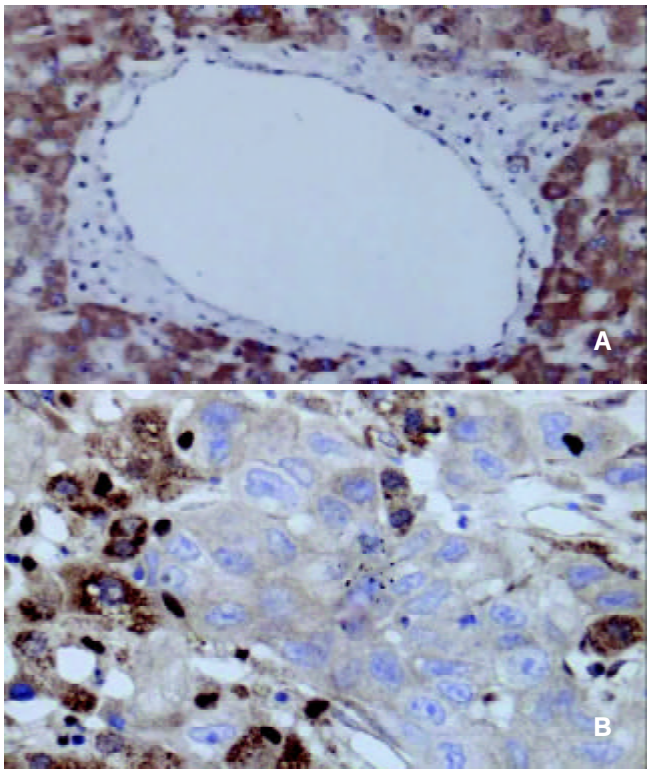

Figure 1 (A) Strong positive staining of Fhit in normal liver tissue SP×100 (B) Negative staining of Fhit in invading hepatocellular carcinoma (upper right) compared with strong positive staining of Fhit in para-neoplastic liver tissue (lower left) (SP×400).

Table 2 Relationship between Fhit expression and clinicopathological indice

	Fhit score			
	n	-	+	++
Histological grade				
Grade I	15	3	4	8
Grade II	39	12	9	18
Grade III	29	14	8	7
Clinical stage				
Stage I-II	37	4	4	29
Stage III-IV	46	25	17	4
Size (mm)				
≤50	39	10	7	22
>50	44	19	14	11

DISCUSSION

Fhit protein is expressed in most types of normal human tissues but has been found to be frequently reduced or lost in a variety of human tumors due to alterations in its gene transcription or gene deletion[1]. It has thus been suggested that FHIT gene is a candidate tumor suppressor gene for multiple carcinomas. Fhit,

the 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 be to catabolize ApppA (Ap_3A) or related substrates. Ap_3A is an analogue 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 contribute to carcinogenesis. Second, the activity of Fhit on mRNA cap analogs raises the possibility that failure of a decapping function might be tumorigenic, however, 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 signaling by Fhit-substrate complexes or compounds as an active form of Fhit, which may be more important than its role of hydrolase. Fourth, Fhit might have a nucleotide-independent role as a tumor suppressor[33].

Yuan *et al*.[30] found that 4 of 9 cell lines and 5 of 10 primary hepatocellular carcinomas did not express Fhit protein or only expressed reduced levels of Fhit. Consistent with their results, we found that 50 of 83 (65.2 %) primary hepatocellular carcinomas showed markedly reduced or loss of expression of Fhit, suggesting that loss of Fhit protein might be related to the carcinogenesis of hepatocytes. Furthermore, decreasing expression of Fhit protein with higher histological grading, and more significantly with advanced clinical stages (stage III and IV) of primary tumors and bigger tumor size (>50 mm in diameter) suggests that loss of Fhit expression is strongly associated with the development and progression of hepatocellular carcinoma. Similar association between loss of FHIT function and the stage, grade and poor prognosis of tumors has been noted in lung cancer[4-5], colorectal carcinoma[18] and advanced breast cancer[8].

In summary, expression of Fhit is reduced or lost in a significant proportion of hepatocellular carcinomas and especially in more advanced stages of primary tumors. Thus, detection of Fhit protein expression by immunohistochemistry in hepatocellular lesions may provide important diagnostic and prognostic information in practical clinical application.

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