[CANCER RESEARCH 61, 4382–4385, June 1, 2001]

Abnormal Fragile Histidine Triad (*FHIT*) Expression in Advanced Cervical Carcinoma: A Poor Prognostic Factor

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ABSTRACT

The *FHIT* gene is a candidate tumor suppressor gene that has been implicated in the development of cervical carcinoma. We hypothesized that abnormal Fhit expression might be a poor prognostic factor for patients with cervical cancer. The tumors from 59 high-risk patients (stage II–III) were evaluated for abnormal Fhit expression by immunohistochemical staining. Abnormal Fhit expression (absent or reduced) was noted in 66% of the specimens. There was no statistical difference with respect to stage, performance status, para-aortic node metastasis, completion of therapy, grade, race, age, and HIV status between the normal and abnormal Fhit expression groups. The 3-year survival for patients whose tumors displayed normal Fhit expression *versus* abnormal Fhit expression was 74% *versus* 37%, respectively. Univariate analysis demonstrated a difference in survival that was statistically significant for age <55 years *versus* ≥55 years ($P = 0.015$), normal Fhit expression *versus* abnormal Fhit expression ($P = 0.015$), and stage II *versus* stage III ($P = 0.033$). Multivariate analysis showed that abnormal Fhit expression was a poor prognostic factor ($P = 0.015$).

INTRODUCTION

There were approximately 12,800 cases of invasive cervical carcinoma in the United States in 2000, resulting in 4,800 deaths (1). The vast majority of these cases were squamous cell carcinomas. Whereas the exact etiology of cervical cancer remains unknown, it appears that an early event is infection by HPV,[2] which has been found in 99.7% of squamous cell cervical carcinomas (2, 3). The role of HPV infection in the development of cervical dysplasia involves the inactivation of cellular proteins p53 and Rb by the HPV proteins E6 and E7 (4). However, not all patients with HPV infection develop cervical dysplasia or invasive cervical carcinoma. This implies that other molecular alterations must accompany the HPV infection for the full development and progression of invasive cervical carcinoma. These other alterations most likely include oncogene activation or tumor suppressor gene inactivation.

LOH on the short arm of chromosome 3 has been detected in up to 75% of cervical carcinomas (5–8). This high rate of LOH suggests that a tumor suppressor gene involved in cervical cancer is located in this region of chromosome 3. The *FHIT* gene, which was identified in 1996, is a candidate tumor suppressor gene located on chromosome 3p14.2 (9). Many studies have reported altered *FHIT* expression in a variety of carcinomas including head and neck, lung, kidney, gastrointestinal, and breast cancer (10, 11). Our laboratory has previously reported altered *FHIT* expression in 68% of cervical carcinoma cell lines (12). Additional studies have confirmed that the *FHIT* gene is abnormally expressed in 30–78% of cervical carcinoma cell lines and primary tumors (13–18). In addition, alteration of Fhit expression occurs early in the development of cervical carcinoma because it can be detected in cervical dysplasia (13, 14). Therefore, the *FHIT* gene, a candidate tumor suppressor gene, may be involved in the development and progression of cervical dysplasia to invasive cervical carcinoma. This study was designed to evaluate whether abnormal Fhit expression, as determined by IHC staining, in high-risk (stage II–III) tumors correlated with patient survival.

MATERIALS AND METHODS

Tissue Specimens and Patient Characteristics. Paraffin-embedded tissue specimens from 59 patients were obtained according to institutional review board-approved protocols at participating institutions. The tissue blocks were catalogued, and 5-μm sections were obtained. One section was stained with H&E and reviewed by a pathologist to confirm the presence of cervical carcinoma.

Patient stage was determined in accordance with the FIGO staging system. The standard treatment for patients with stage II and III squamous cell carcinoma is as follows. Patients with stage II or stage III without para-aortic lymph node involvement were treated with whole pelvic radiotherapy to a dose of 80–95 Gy to point A. Patients with para-aortic nodal involvement by were treated with 44–48 Gy to the para-aortic region in addition to whole pelvic radiotherapy.

Clinicopathological factors including the stage of disease, treatment, patient demographics, grade, race, age at diagnosis, date of last visit, performance status, completion of therapy, disease recurrence, and survival were abstracted from the pathology reports and medical record of each patient. Median follow-up time was 28 months (range, 14–96 months), with a minimum follow-up time for surviving patients of 2 years. Median follow-up times for the normal and abnormal Fhit expression groups are 34.5 and 26 months, respectively.

Immunohistochemistry. The IHC staining for detection of the Fhit expression has been described previously (13). Two authors (T. C. K. and M. J. B.) independently scored the IHC slides as normal or abnormal. Normal Fhit staining was characterized by diffuse, moderate to strong cytoplasmic staining (score, 2+/3+). Abnormal Fhit staining was characterized by no cytoplasmic staining in the tumor cells (score, 0), or decreased intensity of staining (score, 1+) with preserved normal staining in the surrounding stroma and external controls. Staining was repeated on all specimens and independently scored, and in the rare occasion of a mismatch, a third staining was performed. The second observer was blinded to the previous observer's score as well as patient information.

Statistical Analysis. Statistical methods used were Fisher's exact test, the Mann-Whitney test, and univariate and multivariate survival analyses using the likelihood ratio test of the stratified Cox proportional hazards model. Kaplan-Meier survival plots were used to estimate survival at 3 years. All analyses were performed using the SAS Version 8 statistical package.

RESULTS

Fifty-nine patients with invasive squamous cell cervical carcinoma (32 patients with stage II and 27 patients with stage III disease) were included in this study. IHC staining of these specimens for Fhit expression showed intense staining of stromal cells (Fig. 1) and normal cervical epithelium (data not shown). Fig. 1*B* shows normal staining for Fhit in malignant cervical epithelial cells; Fig. 1*D* shows reduced staining (decreased intensity) for Fhit. Negative or absent staining (abnormal) for Fhit is shown in Fig. 1*F* (no staining of cervical cancer cells). For statistical analysis, the patients were arranged into two groups: (*a*) normal Fhit

Received 1/26/01; accepted 4/3/01.

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[2] The abbreviations used are: HPV, human papilloma virus; LOH, loss of heterozygosity; IHC, immunohistochemical; FIGO, International Federation of Gynecology and Obstetrics.

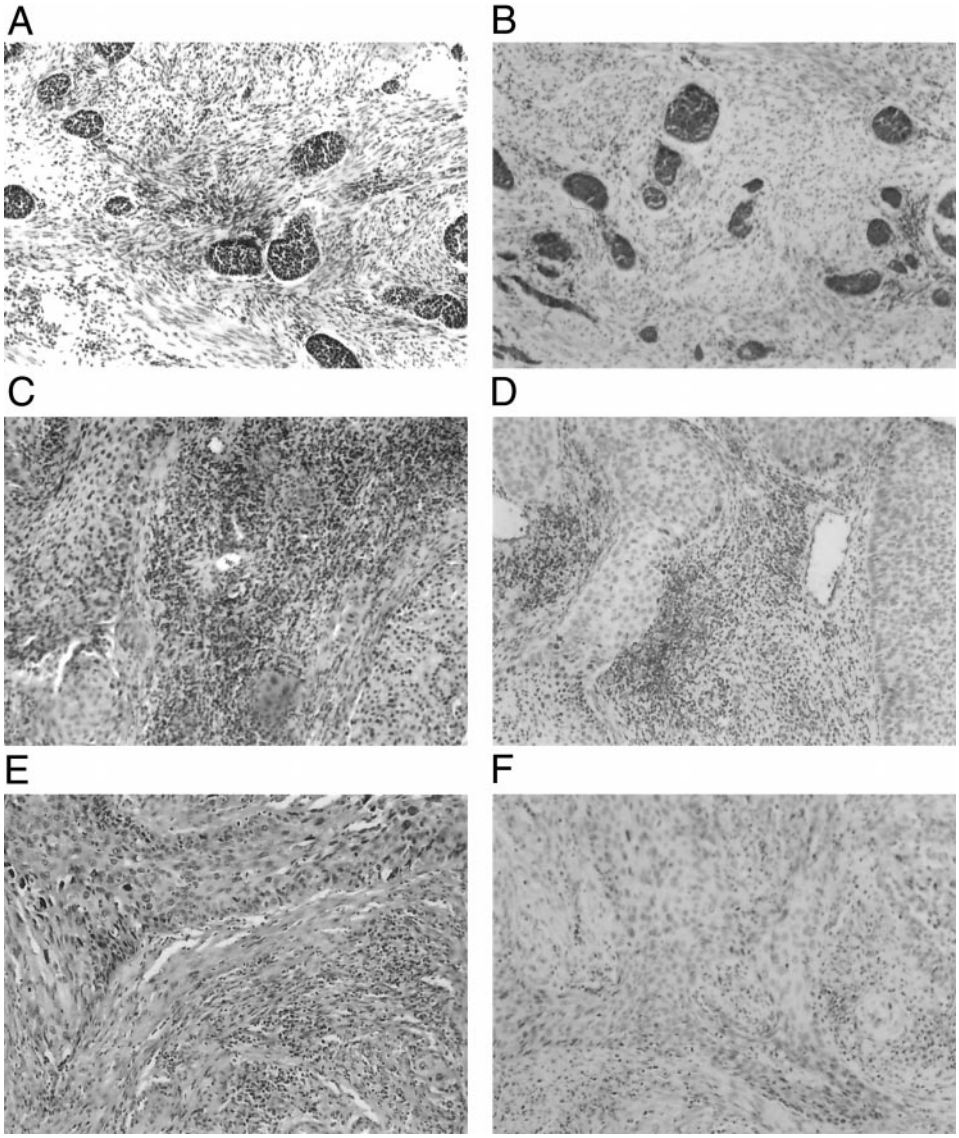

Fig. 1. IHC staining of cervical carcinomas. *A*, H&E staining of invasive cervical carcinomas (*A*, *C*, and *E*). Fhit expression in corresponding sections demonstrates normal (*B*), abnormal (reduced; *D*), and abnormal (negative; *F*) staining.

expression (normal staining); and (*b*) abnormal Fhit expression (reduced and negative staining for Fhit).

There were 39 (66%) patients whose tumors displayed abnormal Fhit expression and 20 patients (34%) with normal Fhit expression. There was no statistical difference between patients whose tumors had abnormal Fhit expression *versus* those that had normal Fhit expression when comparing age, race, para-aortic lymph node metastases, grade, HIV status, performance status, and completion of therapy (Table 1). There was no statistical difference in the incidence of abnormal Fhit expression in stage II patients (23 of 32, 72%) compared to stage III patients (16 of 27, 59%; $P = 0.41$).

Univariate analysis of clinicopathological factors relevant to patient survival revealed three factors that were statistically significant for survival including age ($P = 0.015$), stage ($P = 0.033$), and abnormal Fhit expression ($P = 0.015$; Table 2). The results of the Cox proportional hazard regression analysis are shown in Table 3. When the data were stratified in the multivariate analysis with respect to age and stage, abnormal Fhit expression remained significant with $P = 0.015$. The Cox regression analysis revealed that abnormal Fhit expression was an independent poor prognostic factor in advanced-stage squamous cell cervical carcinoma ($P < 0.05$).

Fig. 2 shows the Kaplan-Meier survival plots of patients whose tumors had abnormal Fhit expression and patients whose tumors had normal Fhit expression; this shows an estimated 3-year survival of 74% for patients with normal Fhit expression and 37% survival for patients with abnormal Fhit expression. Fig. 3*A* shows the Kaplan-Meier survival plot for patients with stage II carcinomas that compared abnormal Fhit expression with normal Fhit expression. The estimated 3-year survivals were 78% for patients with normal Fhit expression compared with 51% for patients with abnormal Fhit expression. Fig. 3*B* shows the Kaplan-Meier survival plot for patients with stage III tumors comparing normal Fhit expression with abnormal Fhit expression. The estimated 3-year survivals were 70% *versus* 19%, respectively.

DISCUSSION

This study was designed to evaluate abnormal Fhit expression as a prognostic marker in patients with high-risk cervical carcinoma. The results showed that the majority of these advanced cervical carcinomas have abnormal Fhit expression. By univariate analysis, abnormal FHIT expression was a poor prognostic variable for survival. Kaplan-Meier survival plots showed that independent of stage and age, patients whose tumors had abnormal Fhit expression had a decreased survival at 3 years (Fig. 2). Multivariate analysis demonstrated that abnormal Fhit expres-

Table 1 *Clinicopathological characteristics of abnormal and normal Fhit expression groups*

	Abnormal Fhit expression (n = 39)	Normal Fhit expression (n = 20)	P
Stage			NS[a]
II	23	9	
III	16	11	
Performance status[b]			NS
0	27	17	
1	11	3	
3	1		
Para-aortic[c]			NS
Negative	31	17	
Positive	8	3	
Grade			NS
1	4	3	
2	20	11	
3	15	6	
Race			NS
African American	21	13	
Caucasian	16	6	
Other	2	1	
HIV			NS
Negative	37	19	
Positive	2	1	
Age (yrs)			NS
Median	52	63.5	
Range	24–86	21–80	
Completion of therapy			NS
Yes	35	20	
No	4	0	

[a] NS, nonsignificant.

[b] Performance status was determined according to FIGO criteria: 0, unrestricted; 1, restricted in strenuous activity; 2, ambulatory >50% of waking hours; 3, ambulatory <50% of waking hours.

[c] Sampling of lymph nodes adjacent to the aorta was done according to FIGO criteria.

Table 2 *Univariate analysis for survival*

Variable	Median survival (months)	P
Stage		
II	52	0.033
III	25	
Fhit		
Abnormal	28	0.015
Normal	74	
Race		
Caucasian	30	NS[a]
African American	41	
Other	74	
Age		
≥55	52	0.014
<55	26	
Grade		
1	41	NS
2	39	
3	30	
Performance status		
0	41	NS
1–3	25	

[a] NS, nonsignificant.

Table 3 *Multivariate analysis*

Variable	Relative risk[a]	P
Stage		
III *vs.* II	2.5 (1.2–5.4)	0.030
Fhit		
Abnormal *vs.* normal	2.8 (1.2–7.8)	0.015
Age (yrs)		
<55 *vs.* ≥55	1.6 (0.78–3.5)	0.20

[a] The 95% confidence interval is shown in parentheses.

sion was an independent poor prognostic factor with a relative risk of 2.8. These findings may allow this molecular alteration to be used as a marker to identify patients who are at high risk for disease recurrence and death.

FHIT expression has been analyzed in multiple epithelial cancers including gastrointestinal, lung, renal, head and neck, and breast carcinomas. In all of these cancers, a subset of tumors displayed altered *FHIT* expression (10). However, the prognostic value of abnormal *FHIT* expression in these tumors remains unclear. Capuzzi *et al.* (19) showed that altered Fhit expression was associated with increasing stage and decreased survival in patients with gastrointestinal carcinomas. In contrast,

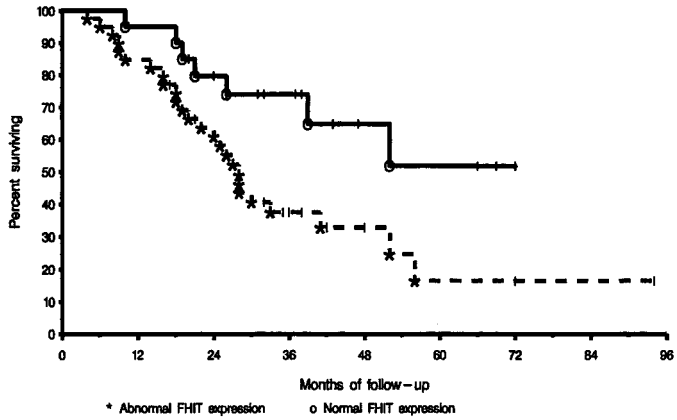

Fig. 2. Kaplan-Meier survival plot by Fhit expression. The *top curve* is the survival of patients whose tumors have normal Fhit expression, demonstrating a 3-year survival of 74%. The *bottom curve* is the survival of patients whose tumors have abnormal Fhit expression, demonstrating a 3-year survival of 37%.

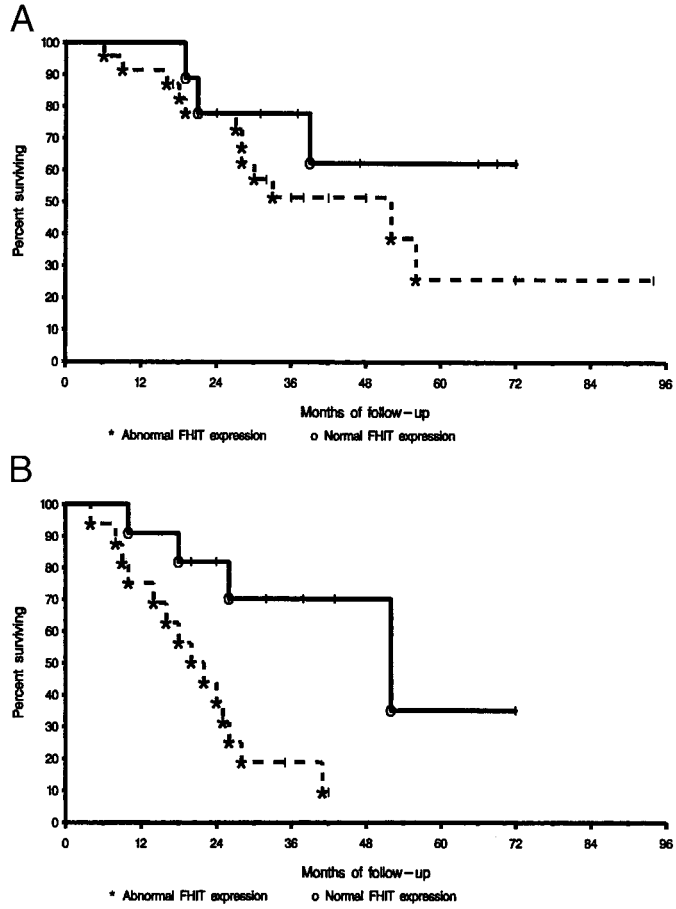

Fig. 3. *A*, Kaplan-Meier survival plot for stage II. The *top curve* is the survival of patients whose tumors have normal Fhit expression, demonstrating a 3-year survival of 78%. The *bottom curve* is the survival of patients whose tumors have abnormal Fhit expression, demonstrating a 3-year survival of 51%. *B*, Kaplan-Meier survival plot for stage III. The *top curve* is the survival of patients whose tumors had normal Fhit expression, demonstrating a 3-year survival of 70%. The *bottom curve* is the survival of patients whose tumors have abnormal Fhit expression, demonstrating a 3-year survival of 19%.

Noguchi *et al.* (20) reported no difference in prognosis or stage between patients with gastric cancer whose tumors showed LOH of the *FHIT* gene and patients whose tumors did not. In esophageal carcinoma, abnormal Fhit expression correlated with stage but did not predict for a poor survival. Two studies examined stage I non-small cell lung carcinomas and demonstrated that patients whose tumors had abnormal *FHIT* expression have a statistically significant decrease in median survival (21, 22). Abnormal Fhit expression has also been demonstrated in breast cancer. Gatalica *et al.* (23) showed that as disease progressed from dysplasia to advanced-stage disease, the loss of Fhit expression becomes more pronounced. This study did not evaluate abnormal Fhit expression as a prognostic indicator for survival. These conflicting studies may reflect a more complex mode of action for *FHIT* or the presence of multiple tumor suppressor genes within the 3p region.

In gynecological malignancies, abnormal *FHIT* expression has been observed in cervical and endometrial carcinomas, but not to a significant degree in ovarian cancer (24–26). In endometrial carcinoma, Segawa *et al.* (24) showed that abnormal Fhit expression was associated with advanced surgical stage and decreased survival but was not an independent prognostic factor. Multiple studies have demonstrated abnormal *FHIT* expression in cervical cancer and its precursor lesions (13, 15–18). Helland *et al.* (26) attempted to correlate the survival of patients with cervical carcinoma to loss of P53, RB1, and Fhit expression. Whereas this study by Helland *et al.* (26) suggested that abnormal Fhit expression may have a role in the development of cervical carcinoma, it did not show a decreased survival in patients whose tumors demonstrated abnormal Fhit expression. These results contrast with our findings, in which abnormal Fhit expression was an independent poor prognostic factor. This difference in results may be due to different inclusion criteria. Helland's study included all histological types and all stages of disease, whereas our study included only stage II and III of the squamous subtype. The utility of abnormal *FHIT* expression may therefore be dependent on histological type and examination of a specific high-risk patient population.

The *FHIT* gene is a candidate tumor suppressor gene, although its precise mechanism of action remains unclear. Reexpression of this gene in tumor cells has yielded conflicting results (27–30). These conflicting data suggest that the mechanisms involved with the function of the *FHIT* gene may be different from those of other classic tumor suppressor genes such as *p53* and *Rb1*. Sard *et al.* (29) reported that the *FHIT* gene is involved in the regulation of the cell cycle and that its tumor suppressor activity derived from proapoptotic activity. However, Otterson *et al.* (27) evaluated the function of the *FHIT* gene and did not discover any regulation of the cell cycle or function with respect to induction of apoptosis. These varied results may reflect that *FHIT* functions in a tissue-specific fashion or at a particular point in the multistage process of carcinogenesis.

Our previous work revealed that a high percentage of cervical cancers and their preneoplastic lesions have alterations in *FHIT* expression. This study extends these results by demonstrating that abnormal Fhit expression was a poor prognostic feature of high-risk cervical cancers. These results are consistent with the hypothesis that this gene has a role in the development and progression of cervical carcinoma.

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