Loss of FHIT Expression in Breast Cancer Is Correlated with Poor Prognostic Markers

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Abstract

Objective: **The fragile histidine triad (*FHIT*) gene is a putative tumor suppressor gene that is thought to be involved in the carcinogenesis of breast cancer. Loss of FHIT expression has been observed in up to 72% of breast cancers and has been associated with increased p53, a high proliferation index, and increased tumor size and grade. However, loss of FHIT expression has not been investigated in association with apoptosis and cyclooxygenase-2 (COX-2) expression in breast cancer. Furthermore, expression of FHIT in primary breast tumors and their metastatic axillary lymph nodes has also not been previously described. The purpose of this study was to evaluate the expression of FHIT, COX-2, bcl-2, and p53 in primary breast tumor tissue; correlate their expression with known clinical and pathologic markers; and in cases when tissue was available, evaluate the expression of FHIT and COX-2 in the corresponding metastatic axillary lymph node in the same patient.**

Methods: **Primary breast tumor specimens from 80 patients were examined for the presence of FHIT, COX-2, bcl-2, and p53 expression by immunohistochemistry using standard methods. When tissue was available, the expression of FHIT and COX-2 was also evaluated in the corresponding metastatic axillary lymph node specimen.**

Results: **FHIT expression in primary breast tumors was 56%. There was a significant correlation between FHIT expression in primary breast tumor and bcl-2 expression (P = 0.017). We also observed a significant inverse correlation between FHIT expression in primary breast tumor tissue and p53 expression (P = 0.023) in lymph node–negative cases. A significant inverse correlation between FHIT expression in the primary tumor and Ki-67 (P = 0.009) was also observed in lymph node–negative cases. FHIT expression in primary tumors correlated with FHIT expression in the metastatic lymph node (52.5%; P = 0.001). FHIT expression in primary tumors did not correlate with COX-2 expression.**

Conclusion: **Our results suggest that loss of FHIT expression in breast cancer is associated with poor prognostic features. Furthermore, loss of FHIT expression is also seen in metastatic axillary lymph node. The prognostic and predictive value of these findings needs to be further evaluated in larger trials with longer follow-up.** (Cancer Epidemiol Biomarkers Prev 2005;14(7):1681–5)

Introduction

The *FHIT* gene, which is located at chromosome 3p14.2 (1), is a candidate tumor suppressor gene in breast and other cancers (2, 3). Tumors that have genomic *FHIT* alterations or altered *FHIT* transcripts usually do not express or express reduced levels of FHIT proteins (4-8). Several studies recently evaluated the alterations in the *FHIT* gene in breast cancer. Loss of heterozygosity within the *FHIT* gene is one of the alterations (9-11). Deletions of the *FHIT* gene have also been observed in preneoplastic lesions (10), suggesting that *FHIT* deletions could be an early event in breast carcinogenesis. Altered transcription is frequently due to internal deletions within *FHIT*, and point mutations are rather rare (4). One of the mechanisms by which loss of expression in breast cancer can occur is due to hypermethylation of *FHIT* (12).

Although altered FHIT transcripts have been reported in 20% to 38% of primary breast carcinomas (2, 6, 13), a reduction or absence of FHIT protein can be seen in up to 72% of breast carcinoma samples (6). The exact clinicopathologic significance of loss of FHIT expression in breast cancer is not known; however, several studies have indicated that it can be associated with poor clinical outcome (6, 14-16). Furthermore, the relationship between FHIT expression and other potential prognostic markers in breast cancer is not known. One if the other marker is the cyclooxygenase-2 (COX-2) enzyme, which is believed to play an important role in breast carcinogenesis. COX-2 expression has been shown in up to 88% of invasive breast cancers (17-20) and is associated with poor clinical outcome (21).

To clarify further the role of FHIT expression in breast cancer and its relation to other prognostic markers, we evaluated the expression of FHIT, COX-2, bcl-2, and p53 in primary breast tumor tissue of 80 patients with the diagnosis of breast cancer and correlated their expression with known clinical and pathologic markers. When tissue was available, the expression of FHIT and COX-2 were also analyzed in the corresponding metastatic lymph nodes in the same patient.

Materials and Methods

Patients and Tumor Specimens. Formalin-fixed and paraffin-embedded primary breast tumor tissue blocks from 80 patients with breast cancer who were seen and operated on at the University of Texas Southwestern Medical Center were examined for the purpose of the study. When tissue was available from the same patient, metastatic axillary lymph node was examined as well. Information about the patients' clinical history was obtained from the patients' medical records. The age at the time of diagnosis was considered the patient's age. The size of the primary breast tumor was considered the largest tumor diameter observed after surgical excision. Lymph node status was determined with histologic evidence of metastatic breast carcinoma.

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Table 1. Patient characteristics

Characteristic (n = 80)	No. patients (%)
Age (median)	51
≤50	33 (41)
>50	44 (55)
Unknown	3 (4)
Menopausal status	
Premenopausal	42 (52)
Postmenopausal	30 (37.5)
Unknown	8 (10)
Tumor size (cm)	
<2	34 (42)
>2	43 (53)
Unknown	3 (4)
Lymph node status	
Negative	19 (24)
Positive	61 (76)
1-3	25 (31)
≥4	34 (42)
Unknown	2 (3)
ER status	
Positive	52 (65)
Negative	28 (35)
PR status	
Positive	41 (51)
Negative	39 (48)
Tumor stage	
I	11 (14)
II	43 (54)
III	20 (25)
IV	5 (6)
Unknown	1 (1)

Patient characteristics are shown in Table 1. The median age of the patients was 51 years. Sixty-five percent (52 of 80) of the tumors were estrogen receptor (ER) positive and 35% (28 of 80) were ER negative. Fourteen percent (11 of 80) of patients had stage I disease, 54% (43 of 80) stage II, 25% (20 of 80) stage III, and 6% (5 of 80) stage IV.

Immunohistochemistry. All immunostaining was done at room temperature and carried out on the Ventana Nexes IHC Automated Staining Module (Ventana Medical Systems, Tucson, AZ). Staining for p53 was done using p53 Predilute (Ventana Medical Systems), bcl-2 using bcl-2 Predilute (Ventana Medical Systems), FHIT using Zymed Laboratories, (San Francisco, CA) COX-2 using Cayman Chemical, Co. (Ann Arbor, MI), HER-2/*neu* using DAKO (Carpinteria, CA). Reagents were used as supplied in the Ventana Basic Detection Kit. Heat-induced epitope retrieval citrate buffer solution (pH 6.0) was obtained from Zymed Laboratories. Optimum primary antibody dilutions were predetermined using known positive control tissues. A known positive and negative control section was included in each run to assure proper staining. Sections were incubated in a freshly prepared mixture of diaminobenzidine and H_2O_2 and were counterstained with hematoxylin, dehydrated in a graded series of ethanols and xylene, and coverslipped. Both the extent and intensity of immunopositivity were considered when scoring FHIT protein expression. The extent of positivity was scored as 0, negative; 1+, weak intensity, <30% of cells staining; 2+, moderate intensity, 31% to 60%; and 3+, intensity as strong as positive control, >60%. For statistical analysis, diffuse absence of staining was regarded as loss of FHIT expression, whereas any level of staining, regardless of percentage of cells staining, was considered positive for FHIT expression.

Statistical Analysis. Patient age, tumor size, and the number of positive lymph nodes were analyzed as continuous variables. Fisher's exact test or the χ^2 test was used to examine the relationships among categorical variables. Student's t test was used to test for differences in age, and the nonparametric Wilcoxon test was used to test for differences in the number of positive lymph nodes and tumor size. Spearman correlation coefficient testing was done to correlate the expression of bcl-2, p53, COX-2, and FHIT in the primary breast tumor versus the lymph node. Ps < 0.05 were considered statistically significant; all P values were two sided.

Results

FHIT Expression. According to the criteria for immunohistochemistry evaluation, FHIT expression was observed in 45 of 80 (56%) primary breast tumor tissue samples (Fig. 1A). In 59 cases, primary breast tumor tissue and corresponding metastatic axillary lymph node from the same patient was available, and FHIT expression was observed in 38 of the 59 (52.5%) metastatic axillary lymph nodes (Fig. 1B). FHIT expression in primary breast tumor tissue correlated with FHIT expression in metastatic lymph node (r_s = 0.416, P = 0.001, n = 59). This was observed in ER negative (r_s = 0.577, P = 0.007, n = 19) as well as in ER-positive cases (r_s = 0.358, P = 0.014, n = 40).

Correlation between FHIT Expression and Other Markers and Clinicopathologic Variables (Tables 2 and 3). COX-2 expression was found in 88% (37 of 42) of the primary breast tumor tissue (Fig. 2A) specimens and did not correlate with FHIT expression. COX-2 expression in the lymph node was 83% (20 of 24; Fig. 2B). Primary breast tumor tissue and corresponding metastatic lymph node from the same patient was available in 23 cases. In those cases, COX-2 expression in primary breast tumor tissue correlated with COX-2 expression in the metastatic lymph node (r_s = 0.402, P = 0.028, n = 23).

No significant correlation was found between tumor or lymph node COX-2 expression and other pathologic markers: FHIT, HER-2/*neu*, p53, bcl-2, tumor size, number of lymph nodes, or stage. The only significant finding was that increased lymph node COX-2 expression was inversely correlated with age (<50 years; r_s = −0.343, P = 0.04, n = 23).

Bcl-2 expression was observed in 44 of 80 (55%) primary breast tumor tissue samples. There was a significant correlation between FHIT expression in primary tumor and bcl-2 expression (r_s = 0.237, P = 0.017, n = 80). When lymph node–positive and –negative cases were analyzed separately, we found that this correlation was significant only in lymph node–negative cases (r_s = 0.419, P = 0.047, n = 19).

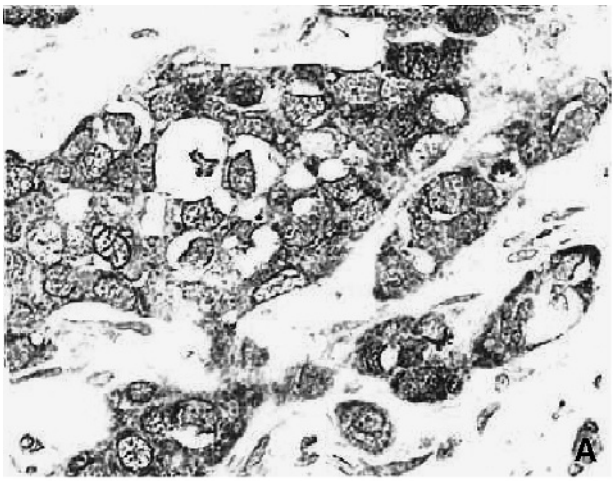
P53 expression was found in 71% (57 of 80) of the primary breast tumor tissues. There was a significant inverse correlation between FHIT expression and p53 expression in the primary breast tumor (r_s = −0.477, P = 0.023, n = 19) in lymph node–negative cases.

There was a significant inverse correlation between FHIT expression and Ki-67 in primary tumor (r_s = −0.499, P = 0.009, n = 19) in lymph node–negative cases. There was no significant correlation in lymph node–positive cases. FHIT expression in the metastatic lymph nodes was associated inversely with increased Ki-67 (r_s = −0.197, P = 0.061, n = 58).

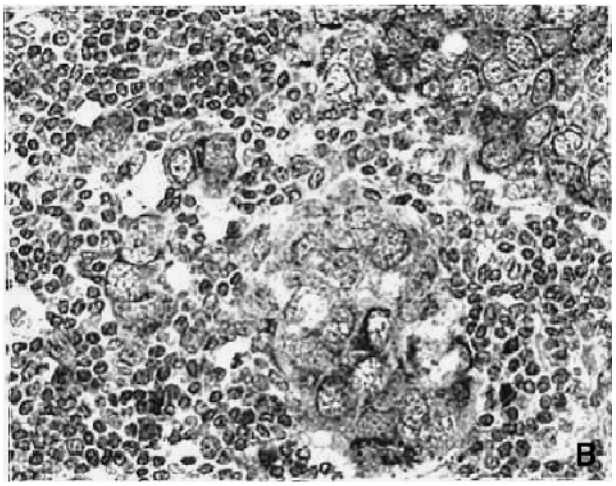
Discussion

The *FHIT* gene, which belongs to the histidine triad superfamily, has been mapped to p14.2 of human chromosome 3 (1). It encodes a cytoplasmic protein that has diadenosine triphosphate activity (22). The most common fragile site of the genome, FRA3B, maps within the *FHIT* gene (1). This fragile site makes FHIT susceptible to rearrangements induced by a variety of carcinogens.

The *FHIT* gene is thought to have tumor suppressor activity. Croce et al. showed that FHIT cDNA-transfected



FHIT - primary



FHIT - mel

Figure 1. A. Immunohistochemical staining of FHIT in primary breast tumor. **B.** Immunohistochemical staining of FHIT in metastatic lymph node.

tumor cells lost tumorigenicity when injected into nude mice (23). It has been postulated that FHIT is involved in breast carcinogenesis. Gatalica et al. showed that loss of FHIT expression was evident in preneoplastic lesions such as hyperplastic and that loss of expression increased during multistep carcinogenesis (14). In our study, reduced FHIT expression was observed in 44% of breast cancer specimens. Although not exactly known, there are several mechanisms by which reduced FHIT expression can occur, such as loss of heterozygosity, deletions at the *FHIT* gene, hypermethylation, abnormal transcripts, and reduced mRNA expression. Loss of heterozygosity at the *FHIT* locus has been detected in breast cancer (3, 11, 24). Homozygous deletions at chromosome 3p14 have also been found in breast cancer and benign proliferative breast diseases (25, 26). Abnormal FHIT transcripts in breast cancer have been reported (3, 10). In one study, the rate of abnormal transcripts was 38% (13). Hypermethylation in the FHIT promoter region has been reported in 31% of breast cancers (12). And finally, reduced FHIT protein expression has been reported in 42% to 72% of breast cancer cases (6, 9, 14, 15).

In our study, we correlated the expression of FHIT with other prognostic markers (COX-2, bcl-2, p53, and HER-2/*neu*) as well as known clinical and pathologic variables. Loss of

Table 2. Association of FHIT expression with clinical and pathologic markers

Features	Total	FHIT		*P*
		Negative	Positive	
Age (y)				0.817
≤50	33	15	18	
>50	44	18	26	
Tumor size (cm)				0.256
<2	34	12	22	
≥2	43	21	22	
PR status				0.013
Positive	41	12	29	
Negative	39	23	16	
ER status				0.815
Positive	52	22	30	
Negative	28	13	15	

FHIT expression was correlated with poor prognostic markers in cases of lymph node–negative breast cancer, such as increased expression of p53 and Ki-67 and decreased expression of bcl-2. Indeed, loss of FHIT expression has been previously correlated with poor prognostic markers such as increased tumor size and grade, ER negativity, and increased p53 expression. Campligio et al. reported loss of FHIT expression in 69% of breast cancer cases (n = 185), and this was associated with increased tumor size and S-phase fraction (6). Yang et al. evaluated and found loss of FHIT expression in 42.2% of breast cancer cases (n = 166), which was associated with increased histologic grade, ER negativity, increased tumor proliferation index, and as our results indicated, increased p53 expression (15). The investigators also reported a trend toward decreased disease-free survival. Thus far, no studies have evaluated the association with bcl-2, COX-2, or HER-2/*neu*. In this study, we showed that FHIT expression does not correlate with HER-2/*neu* expression but is still a poor prognostic marker.

In this study, we showed a significant correlation between loss of FHIT expression and progesterone receptor (PR)–negative status in the primary tumor and in lymph node–negative cases. Costa et al. have previously suggested that PR might have a stronger prognostic role than ER in patients with breast cancer and that PR-negative patients might have a worse outcome than patients with PR-positive disease (27). This substantiates our hypothesis that loss of FHIT expression might be associated with poor prognostic features such as PR negativity. Ingvarsson et al. also evaluated the association between hormonal status and FHIT expression and reported that loss of heterozygosity at the *FHIT* locus in 239 breast cancer patients was associated with ER and PR negativity and

Table 3. Association of FHIT expression with other markers

	Total	FHIT		*P*
		Negative	Positive	
COX-2				0.237
Positive	37	16	21	
Negative	5	3	2	
Bcl-2				0.017
Positive	44	15	29	
Negative	36	20	16	
P53 (LN-negative cases)				0.023
Positive	13	5	8	
Negative	6	1	5	
Ki-67 (LN-negative cases)				0.009
Positive	11	6	5	
Negative	8	0	8	

Abbreviation: LN, lymph nodes.

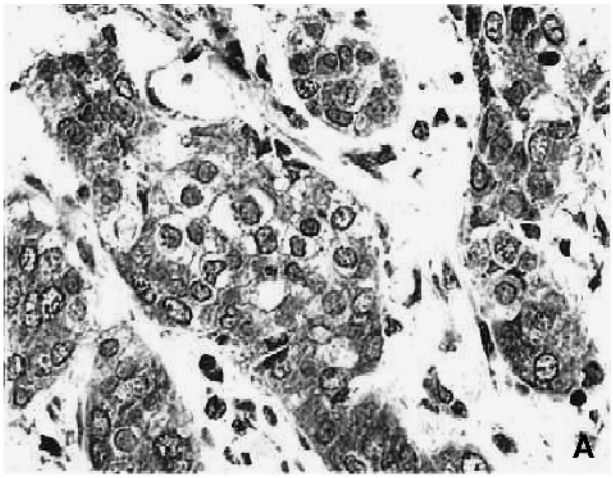


Figure 2. A. Immunohistochemical staining of COX-2 in primary breast tumor. **B.** Immunohistochemical staining of COX-2 in metastatic lymph node.

increased S-phase fraction (16). Hayashi et al. reported abnormal transcripts of the *FHIT* gene in 61 breast cancer specimens and found no association between abnormal FHIT transcript and age, tumor size, nodal status, local recurrence potential, or family history or lifestyle (13). The only significant association was the incidence of bilateral breast cancers. Galatica et al. reported loss of FHIT expression in 72% of breast cancer cases (n = 50); but again, no association was found between FHIT expression and age, tumor grade, tumor stage, and family history (14).

Our finding that loss of FHIT expression was seen in early, lymph node–negative breast cancer cases and correlated with other markers of poor prognosis might indicate that a subset of early breast cancers might behave more aggressively. Obviously, the prognostic value of this finding needs to be validated in larger studies with longer follow-up.

We further showed that FHIT expression in the primary tumor correlated with FHIT expression in the lymph node metastasis, an observation that has not been previously reported. This implies that the phenotypic characteristic of these metastatic tumor cells is preserved. Again, the clinical significance of this finding needs to be evaluated further.

In our analysis, we did not find a significant association between FHIT and COX-2 expression in breast tumor tissue, although we showed that COX-2 expression in the tumor correlated with COX-2 expression in the metastatic lymph node. COX-2 expression has been shown previously to correlate with poor prognostic markers and outcome (21, 28). It was interesting that increased COX-2 expression in lymph nodes was associated with younger patient age (<50 versus ≥50 years).

We also found that loss of FHIT expression correlated with decreased bcl-2 expression.

Decreased bcl-2 expression has been shown to correlate with increased p53 and Ki-67, again implying that loss of FHIT expression is associated with markers of poor prognosis. Although its mechanisms are not yet well explained, a recent study has shown that FHIT is also involved in the regulation of apoptosis and in cell cycle control (29) and its association with bcl-2 needs to be further investigated.

Yang et al. reported that loss of FHIT expression conferred a trend toward decreased disease-free survival in multivariate analysis (15). Ingvarsson et al. also found an association with decreased survival (16). In our study, because the follow-up was short and there were not enough events, and more importantly, because patients adjuvant treatments were not homogenous, survival analysis was not done.

In conclusion, FHIT is involved in breast carcinogenesis and frequent allelic loss at the *FHIT* gene in various malignancies, including breast cancer, implies that FHIT may be a tumor suppressor gene. We have shown that loss of FHIT protein expression is associated with other markers of poor prognosis and that loss FHIT expression is also maintained in the metastatic axillary lymph node in patients with breast cancer. The clinical significance of these findings should be further evaluated in larger cohorts with longer follow-up to evaluate the effect of these findings on disease-free and overall survival.

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