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 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.

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.
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
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 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. Campiglio 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

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

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Table 3. Association of FHIT expression with other markers

Abbreviation: LN, lymph nodes.
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.

References
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