[CANCER RESEARCH 59, 3866–3869, August 15, 1999]

Advances in Brief

FHIT Loss of Function in Human Primary Breast Cancer Correlates with Advanced Stage of the Disease[1]

Manuela Campiglio, Yuri Pekarsky, Sylvie Menard, Elda Tagliabue, Silvana Pilotti, and Carlo M. Croce[2]

Kimmel Cancer Institute, Thomas Jefferson University [M. C., Y. P., C. M. C.], Philadelphia, Pennsylvania 19107, and Oncologia Sperimentale E, Istituto Nazionale per lo Studio e la Cura dei Tumori [M. C., S. M., E. T., S. P.], 20133 Milan, Italy

Abstract

The *FHIT* gene, encompassing the FRA3B fragile site at chromosome 3p14.2, is a tumor suppressor gene involved in different tumor types. We have assessed 29 human primary breast carcinomas for both the presence of abnormal *FHIT* transcripts and the Fhit protein levels as compared with the normal breast epithelium of the same patients. In addition, we have also examined a second retrospective series of 156 consecutive breast carcinomas for the expression of the Fhit protein. In nine (31%) cases of the first series, *FHIT* transcripts were either aberrant or absent as determined by reverse transcription-PCR, and Fhit protein levels in tumors were low or absent as determined immunohistochemically. In 11 other cases (38%), only normal *FHIT* transcripts were detected by PCR, paralleled by the reduction or absence of Fhit protein. In the remaining nine cases (31%), the presence of the normal *FHIT* transcript corresponded to protein levels that were similar in tumor and normal breast epithelia. Thus, alterations in *FHIT* transcripts were detected in 31% of the patients, but reduction or absence of Fhit protein occurred in 69% of the breast carcinoma samples examined. These data suggest that alteration in Fhit expression in breast carcinomas is a frequent event. Analysis of correlation between Fhit expression and pathological, clinical, and biological parameters in these 29 tumors and in a second retrospective series of 156 consecutive primary breast carcinomas indicated that a decrease or an absence of Fhit protein expression is associated with high proliferation and large tumor size.

Introduction

Breast cancer is still the most frequent malignancy among women of western countries (1). The genetic abnormalities that occur in breast cancer involve multiple genetic alterations, which are responsible for the progression from normal breast epithelia to invasive cancer cells.

Chromosomal region 3p14.2 is a frequent target for genetic and cytogenetic alterations in a wide range of solid tumors (for review see Ref. 2), leading to the search for a tumor suppressor gene in this region. The tumor suppressor gene *FHIT*, located at chromosome 3p14.2, is more than 1 Mb in size and encodes a 1.1-kb cDNA with 10 small exons; exon 5 is the first protein coding exon and is flanked in intron 4 and intron 5 by the most common fragile site in the human genome, FRA3B (3). The *FHIT* gene belongs to the histidine triad (4) superfamily and encodes a cytoplasmic M_r 16,800 protein with diadenosine triphosphate (Ap3A) hydrolase activity. The conserved histidines are required for full enzymatic activity (4). The gene is inactivated by deletions in cancer-derived cell lines and primary tumors of the lung (5), head and neck (6), breast (7), stomach and colon (3), and other organs.

Received 2/19/99; accepted 7/1/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by NIH Grant CA39860 and by AIRC (Associazone Italiana per la Ricera sul Cancro).

[2] To whom requests for reprints should be addressed, at Kimmel Cancer Institute, Thomas Jefferson University, Room 1050 BLSB, 233S 10th Street, Philadelphia, PA 19107. Phone: (215) 503-4645; Fax: (215) 923-3528.

Analysis of *FHIT* gene in a series of human primary breast cancer revealed allelic loss in 25% (7) and abnormal transcripts in approximately 30% of the cases (7, 8). *FHIT* homozygous deletions in samples with 3p14.2 aberrations were also found in the benign breast lesions of two women with familial predisposition to breast cancer (9). Another study of normal breast epithelium, breast preneoplastic lesions, and invasive tumors reported the loss of heterozygosity of the *FHIT* locus in two patients with intraductal hyperplasia (10). Studies correlating *FHIT* gene alterations with its protein level are required to determine the grade of inactivation of the gene in breast cancer disease. To address this question, we analyzed 29 cases of primary breast tumors for normal and abnormal *FHIT* transcripts and for the level of expression of Fhit protein in the normal breast epithelia and breast epithelia tumor of the same patient. Down-modulation or absence of Fhit protein was also evaluated in a series of 156 consecutive patients with primary breast carcinomas. In both groups, Fhit protein levels were reduced or absent in almost 70% of the breast cancer samples, whereas aberrant *FHIT* transcripts were detected in only 31% of the samples. Moreover, down-regulation of Fhit protein expression is associated with highly proliferative and large tumors.

Materials and Methods

Patients. The study included two series of primary breast carcinoma obtained from patients surgically treated at Istituto Nazionale Tumori (Milan, Italy). The first (Series N. 1) included 23 DCs[3] and 6 LCs selected for the availability of fresh tumor specimens. The second (series N. 2) included 156 consecutive cases of ductal, lobular, or mixed infiltrating breast cancer, retrospectively examined. Primary tumor diameter and axillary nodal status were obtained from histopathological reports. Histological grading was performed according to Pereira *et al.* (11), based on tubule formation, nuclear morphology, and number of mitoses. Hormone receptors expression was determined by the DCC adsorption technique (12).

RNA Extraction and RT-PCR. Tumor specimens were frozen immediately after surgical resection. Total RNA was extracted from the frozen tumors using RNA-zol (Tel-Test, Friendswood, TX) according to the manufacturer's instructions.

cDNA was synthesized from 1 μg of total RNA. RT was performed in a 20-μl volume of 1× first-strand buffer (Life Technologies, Inc.), 10 mM DTT, 500 μM each dNTP, 0.3 mg/ml random primer (Life Technologies, Inc.), and 300 units of SuperScript II reverse transcriptase (Life Technologies, Inc.). Samples were incubated at 70°C for 5 min and then at 42°C for 1 h before 1 μl of RNase A was added for 30 min.

One μl of cDNA was used for the first PCR amplification with primers EX2F and 06 (CTTTGAAGCTCAGGAAAG and CTGTGTCACTGAAAGTAGACC, respectively) from *FHIT* exons 2–9 in a volume of 25 μl containing 20 pmol of each primer, 200 μM each dNTP, 1× reaction buffer (Boehringer-Mannheim), and 1.25 units Taq polymerase (Boehringer-Mannheim). PCR cycling conditions were: (*a*) initial denaturation at 95°C for 3 min followed by 28 cycles of 30 s at 94°C; (*b*) 30 s at 57°C and 2 min at 72°C; and (*c*) final extension at 72°C for 5 min, using a Perkin-Elmer-Cetus PCR Thermocycler.

[3] The abbreviations used are: DC, ductal carcinoma; LC, lobular carcinoma; RT, reverse transcription.

Table 1 *Summary of tumors, FHIT transcripts, and Fhit protein*

Patients	Histopathology[a]	Protein[b]	RT-PCR	Aberrant products deletions, insertions
1	DC	N	Normal	
2	DC	S	Normal	
3	LC	N	Normal	
4	LC	N	Normal	
5	DC	W	Normal	
6	DC	N	Aberrant	Insertion 65 bp
7	DC	N	Normal	
8	DC	W	Normal + aberrant	Exons 5–8 deletion
9	DC	N	Absent	
10	DC	S	Normal	
11	DC	N	Absent	
12	DC	S	Normal	
13	DC	S	Normal	
14	LC	W	Normal + aberrant	Exons 5–8 deletion
15	DC	W	Normal + aberrant	Exons 4–7 deletion
16	LC	S	Normal	
17	DC	W	Normal	
18	DC	S	Normal	
19	DC	W	Normal	
20	LC	W	Normal	
21	DC	S	Normal	
22	DC	W	Normal	
23	DC	N	Normal	
24	DC	N	Normal + aberrant	Exons 5–7 deletion + insertion
25	DC	S	Normal	
26	DC	W	Normal + aberrant	Exons 4–8 deletion
27	DC	N	Normal + aberrant	Exons 4–6 deletion
28	LC	N	Normal	
29	DC	S	Normal	

[a] Histological types of human breast carcinomas.
[b] Expression of Fhit protein evaluated by immunohistochemistry: N, negative; W, weak; S, strong.

Amplified products were diluted 20-fold and 1 μl was used for a second PCR amplification with primers UR5 and 752R (CTGTAAAGGTCCGTAGTG and CTGCCATTTCCTCCTCTGAT, respectively) from *FHIT* exons 3–9. The nested PCR amplification was carried out for 22 cycles under the same conditions as the first PCR, and products were resolved on a 1.3% agarose gel.

DNA Sequencing. DNA bands corresponding to the normal and abnormal size *FHIT* transcripts were excised from the gel, purified using the Quick Gel extraction kit (QIAGEN), and sequenced on the Applied Biosystems model 373A and 377 DNA sequencers.

Immunohistochemistry. Immunoperoxidase assay was carried out on paraffin-embedded sections of primary breast carcinomas. Briefly, 1–2 μm consecutive sections of formalin-fixed, paraffin-embedded tissue were cut and mounted in poly-L-lysine (Sigma, St. Louis, MO) coated-slides, deparaffined in xylene, and rehydrated in grade alcohol. Endogenous peroxidase activity was blocked by treatment with 0.3% hydrogen peroxide in methanol for 30 min. Fhit antigen enhancement was performed by staining the sections at 120°C in 10 mmol/liter sodium citrate buffer (pH 6.0) for 2 min. After treatment with normal goat at 1:50 for 30 min at room temperature, slides were incubated overnight with different antibodies. Antibodies reactivity was detected using biotinylated goat antirabbit IgG (Dako, Glostrup, Denmark) followed by incubation with streptavidin-conjugated horseradish peroxidase (Dako), and peroxidase activity was detected by amino-ethyl carbazole (AEC). Staining without antibody was performed as a negative control.

The following panel of monoclonal antibodies was applied: (*a*) rabbit polyclonal antibody anti-GST Fhit serum (1:4000); (*b*) anti-c-erbB-2 MAbcB11 (1:60; Ylem, Avezzano, AQ, Italy); (*c*) anti-p53 MAb DO7 (1:500; Novocastra, Newcastle-upon Tyne, United Kingdom); (*d*) anti-bcl2 Mab 100 (1:20; a kind gift from Dr. David Mason, John Radcliffe Hospital, Headington, Oxford, United Kingdom); and (*e*) anti-progesterone receptor MAb1A6 (1:20; DBA, Segrate, MI, Italy) evaluated as described previously(13). Fhit immunostaining was assessed based on cytoplasmic labeling.

Statistical Analysis. χ^2 analysis was used to evaluate differences in frequencies of the various parameters.

Results

Aberrant *FHIT* Transcripts in Human Primary Breast Cancer. Nested RT-PCR analysis of 29 primary breast tumors (23 DCs and 6 LCs) and normal breast epithelium (as a control) using primers within *FHIT* exons 3 and 9 revealed aberrant transcripts in 7 cases and absence of *FHIT* products in 2 cases (Table 1; Fig. 1). Sequence analysis of the aberrant transcripts showed exon deletions involving exon 5 (containing the initiating methionine) in 6 cases—specifically, deletions of exons 4–7 (sample 15), exons 5–8 (samples 8 and 14), exons 4–8 (sample 26), exons 4–6 (sample 27), and exons 5–7 (sample 24). All of the 6 cases also showed the normal-sized fragment of 577 bp, with the correct *FHIT* sequence. Sample 6 showed a larger abnormal *FHIT* transcript in the absence of the normal *FHIT* product; sequence analysis revealed a 65-bp insertion between exon 4 and 5 that leaves the coding region intact. Besides the normal and deleted *FHIT* transcripts, a larger band was detected in samples 2, 22, and 24 and indicated an ~100-bp insertion between noncoding exons 3 and 4 of sample 24, whereas the products were not resolved in sample 2 or 22. In samples 9 and 11, *FHIT* transcripts were not detected.

Expression of Fhit Protein in Human Breast Cancer *versus* Normal Epithelium. Immunohistochemical analysis of formalin-fixed and paraffin-embedded breast tumor and normal epithelium sections of the first series served to define three groups of tumors based on the reactivity of anti-Fhit polyclonal antibody (Table 2). The first group of the first series included nine tumors in which immunostaining was as intense as in the matched normal breast epithelium (*e.g.*, Fig. 2*a*). This cytoplasmic staining was uniform and "strong" (Fig. 2*b* and 2*c*). All 9 cases displayed only the normal-sized Fhit transcript (Table 1).

The second group included nine cases characterized by homogeneous reactivity on the tumor, but immunostaining intensity was much lower than in normal epithelial cells in the same sample (Fig. 2, *d* and *e*); five of these cases showed only normal *FHIT* transcripts by RT-PCR, whereas the other four samples showed both normal and abnormal transcripts (deletions of exons 5–8, 4–7, and 4–8; Table 1).

The third group included 11 cases negative for Fhit protein expression in the tumor, whereas the normal epithelium in the same sample was highly positive (Fig. 2*f*). Six of these tumors displayed normal *FHIT* transcripts. It is possible that the normal RT-PCR band derives from the normal cells contaminating the tumor tissue. Two cases showed a mixture of normal and aberrant transcripts (deletions exon 5–7 and 4–6). In one other sample (sample 6) only an abnormal transcript with an insertion that leaves the coding region intact was revealed; suggesting that this insertion was sufficient to abolish protein expression. In the remaining 2 samples (9 and 11), the absence of Fhit transcripts correspond to the lack of Fhit protein (Table 1). Thus, Fhit protein level were reduced or absent 69% (20 of 29) of the breast carcinoma samples tested.

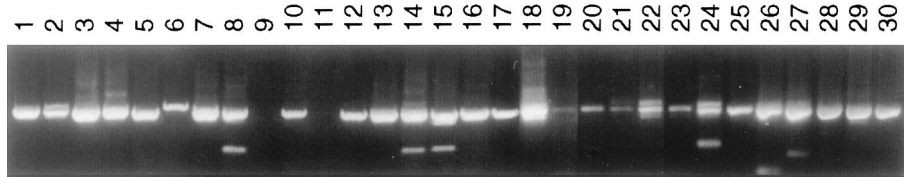

Fig. 1. Expression of *FHIT* transcripts PCR in human primary breast cancer. One μl of cDNA was amplified by nested PCR with primers within exons 3 and 9 in 29 primary breast tumors *(Lanes 1–29)* and 1 normal breast epithelium *(Lane 30)*.

Table 2 *Correlation between FHIT transcripts and Fhit protein expression*

	Protein expression (IHC)[a]		
FHIT transcripts	Strong	Weak	Negative
Normal	9[b]	5	6
Abnormal	0	4	3
Absent	0	0	2
Total	9	9	11

[a] IHC, immunohistochemistry.
[b] Number of patients in each category.

Correlation between *FHIT* Alterations and Bio-pathological Parameters. Reduction and loss of Fhit protein expression was observed with similar frequency also on a retrospective series of 156 consecutive primary breast carcinomas (Table 3).

Analysis of the clinical, pathological, and biological characteristics of tumors in comparison with *FHIT* alterations at the mRNA or protein levels revealed no major differences between tumors with abnormal and normal *FHIT* transcripts. On the other hand, comparison of tumors with strong Fhit *versus* Fhit-negative tumors showed that the latter were more frequently large and poorly differentiated tumors ($P < 0.05$ by χ^2 analysis) based on both high tumor grade and lack of hormone receptor expression. Tumors scored as strongly positive for Fhit protein expression were mainly of small size and more differentiated. Tumors with weak immunostaining were between these two groups. These data were also confirmed on the larger series. It is noteworthy that the series N.2 consisted in smaller tumors, with a lower frequency of poorly differentiated tumors. Nevertheless, the association between loss of Fhit expression and large tumor size was confirmed ($P < 0.05$). In addition, association with a high number of mitoses was also found ($P < 0.05$). The association with tumor grade, found in the first series, was observed only as a trend. No association with HER2/neu overexpression, p53 alteration, and absence of hormone receptors expression was also observed.

Discussion

In the present study, detection of abnormal *FHIT* transcripts was reported in one-third of the human breast carcinomas examined in keeping with our previous data (7). These alterations led to a decrease or absence of Fhit protein in the tumor cells. In addition, down-regulation or absence of Fhit protein was detected in another one-third of the samples displaying normal Fhit transcript. This leads to the conclusion of altered Fhit expression in a larger fraction of tumors (70%) than the one expected on the basis of gene alterations, which suggests that *FHIT* is one of the most frequent targets of alteration in breast cancer. Indeed, well-studied markers of breast carcinomas aggressiveness, such as HER2 and p53 were affected in less than 30% of our cases, as reported previously (13–15). The higher frequency of *FHIT* alteration in human breast cancer suggests the potential usefulness of this marker for prognostic purpose and as a therapeutic target in breast tumors.

In previous studies, loss of function of *FHIT* was attributed to intragenic deletions leading to the production of transcripts incapable of synthesizing the protein (16). In fact, the absence of the

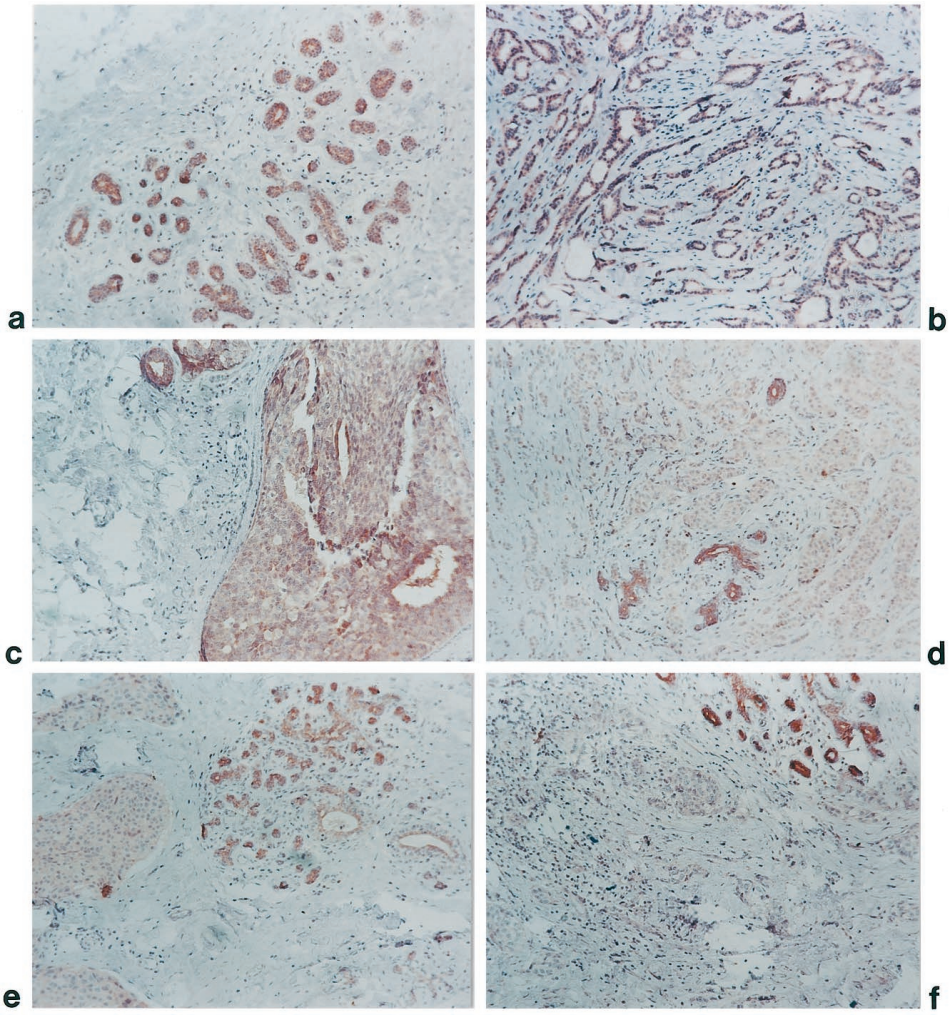

Fig. 2. Expression of Fhit protein in human breast normal and cancer epithelium analyzed by immunohistochemistry on paraffin-embedded breast carcinoma using rabbit GST-Fhit antibody. *a*, strong immunostaining of normal epithelial tissue; *b* and *c*, strong immunostaining of tumor and normal epithelium; *d* and *e*, weak immunostaining of tumor cells and strong immunostaining in normal epithelium; *f*, negative immunostaining of tumor cells and strong immunostaining of normal epithelium.

Table 3 *Clinical, pathological, and biological characteristics of breast carcinomas according to Fhit expression*

	Series 1					Series 2		
	Gene		Protein expression (IHC)[a]			Protein expression (IHC)		
Parameter	Aberrant	Normal	Strong	Weak	Negative	Strong	Weak	Negative
No. of cases	9	20	9	9	11	49	60	47
Age >55 yr	44%	35%	22%	33%	54%	43%	43%	36%
T_2 tumors	67%	67%	44%	67%	90%[b]	32%	46%	52%[b]
High mitosis	NT	NT	NT	NT	NT	37%	55%	64%[b]
Tumor grade III	44%	35%	11%	55%	55%[b]	19%	33%	33%
N+	78%	60%	44%	67%	82%	59%	46%	67%
PgR-neg.	44%	40%	33%	33%	64%	28%	41%	40%
ER-neg.	44%	60%	33%	78%	55%	NT	NT	NT
Nonductal	11%	25%	11%	22%	27%	12%	13%	19%
p53 pos.	22%	20%	22%	20%	18%	30%	15%	17%
neu pos.	33%	25%	33%	20%	27%	22%	33%	23%

[a] IHC, immunohistochemistry; NT, nontested; N+, lymphomal metastases; PgR-neg., negative progesterone receptor expression; ER-neg., negative estrogen receptor expression; pos., positive.
[b] $P < 0.05$ by χ^2 analysis.

initiating methionine located in exon 5 (deleted in some of the samples examined in our study) as well as transfection experiments with constructs sequentially deleted in each *FHIT* coding exon,[4] showed that some deletions did not allow Fhit protein expression. Similarly, sample 6 in our study, which displayed only the abnormal transcript with a 65-bp insertion between exon 4 and 5, did not express detectable amounts of protein, suggesting that even an insertion outside the coding region can abolish Fhit protein synthesis. However, our finding of absent Fhit protein in 11 cases and reduced level of the protein in 4 cases suggests that Fhit protein expression can be abrogated by additional mechanisms. Similarly, Hadaczek *et al.* (17) have reported no *FHIT* gene abnormalities and a very low number of cases with altered RT-PCR products in clear cell renal carcinoma where the great majority of clear cells showed reduced or absent Fhit protein. One possible reason for the lack of correlation between the data by RT-PCR and by immunohistochemistry observed in these 11 cases may rest in the exquisite sensitivity of RT-PCR in detecting very low-abundance transcripts whose products may not be detectable at the protein level; we cannot exclude the possibility that the normal transcript derives from normal ductal cells present in the tumor specimens (less than 1%) that are strongly positive by immunohistochemistry in all of the 11 specimens.

Finally, the decrease or absence of Fhit protein observed in these 29 cases and in a series of 156 consecutive breast carcinomas is clearly associated with a more aggressive disease, inasmuch as it is significantly associated with highly proliferative and large tumors, showing a tendency to be poorly differentiated.

In conclusion, our studies showed for the first time that Fhit inactivation is a very frequent event in breast carcinomas and identifies a more aggressive phenotype.

Acknowledgments

We thank Piera Aiello, Ghirelli Cristina, and Luisa Moiraghi for expert technical assistance.

References

1. Wingo, P. A., Tong, T., and Bolden, S. Cancer statistic. CA Cancer J. Clin., *45:* 8–30, 1995.
2. Kok, K., Naylor, S. L., and Buys, C. H. Deletions of the short arm of chromosome 3 in solid tumors and search for suppressor genes. Adv. Cancer Res., *71:* 27–92, 1997.
3. Ohta, M., Inoue, H., Cotticelli, M. G., Kastury, K., Baffa, R., Palazzo, J., Siprashvili, Z., Mori, M., Mccue, P., Druck, T., Croce, C. M., and Huebner, K. The *FHIT* gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers. Cell, *84:* 587–597, 1996.
4. Barnes, L. D., Garrison, P. N., Siprashvili, Z., Guranowski, A., Robinson, A. K., Ingram, S. W., Croce, C. M., Ohta, M., and Huebner, K. Fhit, a putative tumor suppressor in humans, is a dinucleoside 5′, 5′′′-P1, P3-triphosphate hydrolase. Biochemistry, *35:* 11529–11535, 1996.
5. Sozzi, G., Veronese, M. L., Negrini, M., Baffa, R., Cotticelli, M. G., Inoue, H., Tornielli, S., Pilotti, S., De Gregorio, L., Pastorino, U., Pierotti, M. A., Ohta, M., Huebner, K., and Croce, C. M. The *FHIT* gene at 3p14.2 is abnormal in lung cancer. Cell, *85:* 17–26, 1996.
6. Virgilio, L., Schuster, M., Gollin, S. M., Veronese, M. L., Ohta, M., Huebner, K., and Croce, C. M. *Fhit* gene alterations in head and neck squamos cell carcinomas. Proc. Natl. Acad. Sci. USA, *93:* 9770–9775, 1996.
7. Negrini, M., Monaco, C., Vorechovsky, I., Ohta, M., Druck, T., Baffa, R., Huebner, K., and Croce, C. M. The *FHIT* gene at 3p14.2 is abnormal in breast carcinomas. Cancer Res., *56:* 3173–3179, 1996.
8. Hayashi, S., Tanimoto, M., Hajiro-Nakanishi, K., Tsuchiya, E., Kurosumi, M., Higashi, Y., Imai, K., Suga, K., and Nakachi, K. Abnormal FHIT transcripts in human breast carcinomas: a clinicopathological and epidemiological analysis of 61 Japanese cases. Cancer Res., *57:* 1981–1985, 1997.
9. Panagopoulos, I., Pandis, N., Thelin, S., Petersson, C., Mertens, F., Borg, Å., Kristoffersson, U., Mitelman, F., and Åman, P. The *FHIT* and *PTPRG* genes are deleted in benign proliferative breast disease associated with familial breast cancer and cytogenetic rearrangements of chromosome band 3p14. Cancer Res., *56:* 4871–4875, 1996.
10. Ahmadian, M., Wistuba, I. I., Fong, K. M., Behrens, C., Kodagoda, D. R., Saboorian, M. H., Shay, J., Tomlinson, G. E., Blum, J., Minna, J. D., and Gazdar, A. F. Analysis of the *FHIT* gene and FRA3B region in sporadic breast cancer, preneoplastic lesions, and familial breast cancer probands. Cancer Res., *57:* 3664–3668, 1997.
11. Pereira, H., Pinder, S. E., Sibbering, D. M., Galea, M. H., Elston, C. W., Blamey, R. W., Robertson, J. F. R., and Ellis, I. O. Pathological prognostic factors in breast cancer. IV: should you be a typer or a grader? A comparative study of two histological prognostic features in operable breast carcinoma. Histopathology, *27:* 219–226, 1995.
12. Pellegrini, R., Mariotti, A., Tagliabue, E., Bressan, R., Bunone, G., Coradini, D., Della Valle, G., Formelli, F., Cleri, L., Pierotti, M. A., Colnaghi, M. I., and Ménard, S. Modulation of markers associated with tumor aggressiveness in human breast cancer cell lines by *N*-(4-hydroxyphenyl)retinamide. Cell Growth Differ., *6:* 863–869, 1995.
13. Ménard, S., Casalini, P., Pilotti, S., Cascinelli, N., Rilke, F., and Colnaghi, M. I. No additive impact on patient survival of the double alteration of p53 and c-erbB-2 in breast carcinomas. J. Natl. Cancer Inst., *88:* 1002–1003, 1996.
14. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. Human breast cancer: correlation of relapse and survival with amplification of the *HER-2/neu* oncogene. Science (Washington DC), *235:* 177–182, 1987.
15. Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. Mutations in the *p53* tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res., *54:* 4855–4878, 1994.
16. Huebner, K., Garrison, P. N., Barnes, L. D., and Croce, C. M. The role of the *FHIT/FRA3B* locus in cancer. Annu. Rev. Genet., in press, 1999.
17. Hadaczek, P., Siprashvili, Z., Markiewski, M., Domagala, W., Druck, T., McCue, P. A., Pekarsky, Y., Ohta, M., Huebner, K., and Lubinski, J. Absence or reduction of Fhit expression in most clear cell renal carcinomas. Cancer Res., *58:* 2946–2951, 1998.

[4] Unpublished data.