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Abnormal *FHIT* gene transcript and c-*myc* and c-*erb*B2 amplification in breast cancer★✪

Renata Kowara[1], Filip Gołębiowski[1], Paweł Chrzan[1], Jaroslaw Skokowski[2], Andrzej Karmolinski[3] and Tadeusz Pawełczyk[1, 4]✉

[1]*Department of Molecular Medicine,* [2]*Department of Surgical Oncology,* [3]*Department of Pathomorphology, Medical University of Gdańsk, Gdańsk, Poland;* [4]*Medical Research Center of the Polish Academy of Sciences, Warszawa, Poland*

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Searching for ways to improve the characterization of breast cancer we examined the relationship between the status of the *FHIT* gene transcript and amplification of c-*myc* and the c-*erb*B2 oncogene. Abnormal *FHIT* transcript was detected in 32 of 79 cancers examined. The presence of Fhit protein estimated by Western blots was evident only in cancers exhibiting a normal-sized *FHIT* transcript. This indicates that abnormal *FHIT* transcripts observed in our study did not encode any Fhit protein or the amount of such protein was very low. There was no association between the presence of aberrant *FHIT* gene transcript with age, tumor size, estrogen and progesterone receptor status, local metastases and histological grading. However, the abnormalities in *FHIT* gene transcripts were observed with different frequency depending on the histopathological type of the tumor. The aberrant *FHIT* transcript was detected in 60% of *lobular* cancers and only in 28% of *ductal* cancers. Analyzing the occurrence of c-*myc* and c-*erb*B2 amplification and the presence of aberrant *FHIT* gene transcripts we found that the aberrant *FHIT* transcript more frequently occurred in tissues with c-*myc* amplification. There was a significant ($P < 0.05$) correlation between the occurrence of the aberrant *FHIT* gene transcript with accompanying c-*myc* amplification and positive lymph node status. However, in order to evaluate the predictive value of these findings in breast cancer, an extended clinical follow up will be necessary.

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✉To whom correspondence should be addressed at: Department of Molecular Medicine, Medical University of Gdańsk, Dębinki 7 paw. 29, 80-211 Gdańsk, Poland; tel: (48 58) 349 2750, fax: (48 58) 349 2759, e-mail: tkpaw@amg.gda.pl

The *FHIT* gene on chromosome 3 positioned at 3p14 encodes a protein with the activity of dinucleoside 5′,5′′′-P^1,P^3-triphosphate (Ap_3A) hydrolase (Barnes *et al.*, 1996). Since its discovery in 1996, alterations of the *FHIT* gene have been frequently observed in a variety of tumors (Ohta *et al.*, 1996; Negrini *et al.*, 1996; Fong *et al.*, 1997; Ingvarson *et al.*, 2001; Yuan *et al.*, 2000). Gathered evidence suggests that *FHIT* is a tumor suppressor gene in many cancers. This assumption is supported by the study of Siprashvilli *et al.* (1997), which has showed that transfection of *FHIT* cDNA in cancer cell lines from gastric, large-cell lung, nasopharyngeal and renal cell cancer significantly suppresses tumorigenicity in nude mice. However, in another study, Otterson *et al.* (1998) demonstrated that introduction of wild-type *FHIT* cDNA into a cervical carcinoma cell line lacking endogenous Fhit expression did not change the rate of cell proliferation or altered tumorigenicity in animals. Those contradictory results may suggest the possibility that *FHIT* may exert a tumor suppressor activity in a cell lineage-specific manner or that the *FHIT* gene is indirectly linked with tumor growth. In breast cancer abnormal transcripts of the *FHIT* gene have been observed with various frequency depending on size and histological grade (Hayashi *et al.*, 1997; Pandis *et al.*, 1997; Campiglio *et al.*, 1999). However, it has been reported that clinicopathological analysis of 61 Japanese primary breast cancer specimens revealed no correlation between abnormal *FHIT* transcripts and tumor-node metastasis classification, tumor size, estrogen receptor and progesterone receptor status (Hayashi *et al.*, 1997).

Studies on oncogene amplification in breast cancer have shown prognostic significance of c-*erb*B2 and c-*myc* amplification (Champeme *et al.*, 1994; Berns *et al.*, 1995; Deming *et al.*, 2000). It has been observed that c-*myc* amplification can occur at an early stage of tumor formation and it is not often detectable in nodal metastasis (Watson *et al.*, 1993). In most reports c-*erb*B2 amplification is negatively correlated with estrogen receptor and progesterone receptor status, whereas c-*myc* amplification is more prevalent in the steroid receptor-positive subpopulation. Overall, breast cancer patients with c-*myc* or c-*erb*B2 amplification in their primary tumors have a shorter relapse-free survival and reduced survival (Lonn *et al.*, 1995). In order to evaluate the clinical relevance of *FHIT* gene alterations in breast cancer, we have analyzed *FHIT* transcripts and the amplification of c-*erb*B2 and c-*myc* in primary breast cancer.

MATERIAL AND METHODS

Patients and collection of tissues. Seventy-nine patients with primary breast cancer who attended the Department of Surgical Oncology at the Medical University of Gdańsk (in 1997–1998) were randomly selected for the study. Patients had either an excision biopsy or modified radical mastectomy. All specimens were measured and serially sectioned. All tissues were reviewed by the same pathologist (A.K.). Each specimen was dissected in a 5 mm bread-loaf fashion and measured in all three depicted dimensions. The largest gross dimension was included in the computer analysis. Infiltrating cancers were considered *ductal* or *lobular* or *tubular* based upon their predominant cell type. Grading of invasive tumors followed the modified Bloom and Richardson method (1957). Quantitative estrogen and progesterone receptor analysis (immunohistochemical) was performed on fresh- frozen tissue from the original specimen. Samples of cancerous tissues were dissected by pathomorphologists immediately after surgery, frozen in liquid nitrogen and stored at –80°C.

RNA extraction and reverse transcription. Total RNA was extracted from tissues frozen in liquid nitrogen and stored at –80°C using Total RNA Prep Plus Kit (A&A Biotechnology, Gdańsk, Poland). RNA was stored as a

pellet under ethanol at –40°C. Reverse transcription was performed in 20 μL final volume of 50 mM Tris/HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM dNTPs, 250 ng oligo(dT) (Gibco BRL, Paisley, England), 12.5 U of MMLV-RT (Epicentre Technologies, Madison, WI, U.S.A.), 10 U of RNasin (Promega, Madison, WI, U.S.A.), and 1 μg of RNA. The reaction was incubated for 10 min at 65°C, then run for 90 min at 42°C and boiled for 5 min. In order to control the integrity of the obtained cDNA, a 511-bp fragment of β-actin cDNA was amplified with primers as described (Negrini *et al.*, 1996). The PCR consisted of an initial denaturation at 95°C for 3 min and 35 cycles of 30 s at 95°C, 30 s at 53°C, 1 min at 72°C, and a final extension of 10 min at 72°C. The PCR reaction was performed in a Perkin-Elmer thermal cycler 480.

Nested PCR. To assess the status of the *FHIT* gene transcript we used the nested PCR reaction reported by Ohta *et al.* (1996). The primers used were: UR4, (forward); 7D, (reverse) for the first round and UR5, (forward); 7B, (reverse) for the second round of amplification (Druck *et al.*, 1997).

DNA extraction. DNA extraction from the tissues examined was carried out using Genomic DNA Prep Plus Kit (A&A Biotechnology). The DNA content was measured by light absorption at 260 nm. The purity of DNA was assessed based on the calculated ratio A_{260}/A_{280}.

Amplification of c-myc and c-erbB2. Amplification of c-*myc* and c-*erb*B2 was assessed by a semi-quantitative multiplex PCR assay, which in our hands proved to be useful in evaluating changes in template copy number (Sakowicz *et al.*, 2001; Chrzan *et al.*, 2001). The reaction mixture contained 50 mM Tris/HCl, pH 9.0, 20 mM ammonium sulfate, 100–500 ng of template, 0.50 μM each of 5′ and 3′ primers, 0.25 μM of each dNTP, 2.5 mM $MgCl_2$ and 1 U of Tfl DNA polymerase (Epicentre Technologies). The PCR reaction was performed for 30 cycles of 94°C (1 min), 65°C (1 min), 72°C (2 min) and 94°C (1 min), 56°C (1 min), 72°C (2 min) for c-*myc* and c-*erb*B2 amplification, respectively. For c-*myc* amplification 5′-CTCGAATTCCTTCCAGATATCCTCGCTG-3′ and 5′-CACTGCGCGCTGCGCCAGGTTT-3′ primers were used. This defines a DNA fragment of 258 bp. The c-*myc* primers were based on the nucleic acid sequence of the gene (GeneBank, accession No. J00120). Amplification of c-*erb*B2 was performed with the primers described by Lonn *et al.* (1995). The PCR products were separated by agarose gel electrophoresis and the ethidium bromide-stained bands were quantified with the use of a Gel Doc 2000 system (Bio-Rad) and compared using computer program Quantity One (Bio-Rad). To produce a titration curve of gene amplification we used the cell lines MCF7 and SKBR3 with a defined c-*myc* and c-*erb*B2 gene copy number, respectively. Tissue plasminogen activator (TPA) and thymidine kinase (TK) were the reference genes for c-*myc* and c-*erb*B2 amplification, respectively. The primers for TPA and TK amplification were as described by Lonn *et al.* (1995).

All primers used were from Integrated DNA Technologies, Inc. (Coralville, IA, U.S.A.).

Immunoblots. The examined tissue was homogenized in 3 vol. of 20 mM Hepes/NaOH, pH 7.0, 1 mM EDTA, 0.5 mM Pefabloc SC, 1 μM leupeptin and 0.1 μM aprotinin. Then SDS/PAGE sample buffer was added to the homogenate, and the mixture was boiled for 5 min. The proteins were separated on 12% SDS/PAGE, and electrophoretically transferred to the Immobilon poly(vinylidene-difluoride) (Millipore, Bedford, MA, U.S.A.) transfer membrane. The membrane was blocked with 5% non-fat milk powder in NaCl/P_i with 0.02% NaN_3. Blocked membranes were incubated with rabbit anti-Fhit polyclonal IgG (1:5000 dilution). Immunostaining was performed using alkaline-phosphatase-conjugated goat anti-rabbit IgG, the chromogenic substrate 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

Statistical analysis. The analysis of statistical significance of the correlations between clinicopathological parameters and *FHIT* expression, c-*myc* and c-*erb*B2 amplification was performed using Pearson's chi-square test.

RESULTS

The status of the *FHIT* gene transcript was analyzed in 79 breast cancers by performing RT-PCR. A PCR product of 763 bp, corresponding to the fragment of the *FHIT* gene transcript encompassing exons 2 to 9, was observed in 58% of cancerous tissues (Table I). In addition to the normal-sized product, aberrant size products as well as no products were observed (Fig. 1). The overall occurrence of an aberrant *FHIT* transcript (no transcripts plus abnormal-sized transcripts) was 40.5% (Table 1). The 511 bp fragment of β-actin transcript was successfully amplified in all analyzed tissues (not shown), suggesting a good quality of the cDNA template. Thus, it could be assumed that the observed absence of a PCR product for the *FHIT* gene transcript indicates a real loss of Fhit mRNA expression in these tissues. This assumption was confirmed by Western blots performed on tissue extracts (Fig. 2). The Fhit protein was detected in all cancerous tissues that exhibited the normal *FHIT* transcript. On the other hand, the Fhit protein could not be detected in any of the tissues lacking the *FHIT* gene transcript (compare Fig. 1 and Fig. 2). In all cases in which, besides the normal also the aberrant *FHIT* transcript was present, the Western blot showed only a normal-sized band. This indicates that abnormal *FHIT* transcripts observed in our study did not encode any Fhit protein or the amount of such a protein was very low.

In order to assess the role of *FHIT* alterations in the prognosis of patients with breast

Table 1. Comparison of *FHIT* gene transcript status in breast cancer tissues with some clinical and epidemiological characteristics of patients.

The presence of any abnormal transcript with or without accompanying normally sized transcript was counted as "aberrant". NA, not available.

	FHIT transcript status	
	Normal n = 48	Aberrant n = 32
Histological grading		–
1	1	–
2	31	20
3	5	3
NA	11	9
Lymph node status		
negative	29	17
positive	18	11
NA	1	4
Steroid receptors status		
ER(+)	26	12
ER(–)	16	13
PR(+)	24	11
PR(–)	18	14
NA	6	7
Tumor size (mm)	20.12 ± 8.26	23.44 ± 12.8
Age (years)	58.1 ± 11.3	56.1 ± 10.7

cancer we analyzed the relation between the *FHIT* gene transcript status and other prognostic factors. Clinical and epidemiological characteristics of patients with normal and aberrant *FHIT* transcripts are summarized in Table 1. There was no association of the presence of the aberrant *FHIT* gene transcript with age, tumor size, estrogen and progesterone receptor status, local metastases and histological grading.

In our studies we also analyzed the dependence of *FHIT* transcript status on the histopathological type of tumor. We found that the abnormalities in *FHIT* gene transcripts occurred more frequently in *lobular* cancers (60%) than in *ductal* cancers (28%) (Table 2). The same high occurrence of abnormal *FHIT* transcripts was observed in *tubular* cancers (60%), but we had no sufficient number of patients within this group to perform statistical analysis.

Data from studies on oncogene amplification indicate that in breast cancer amplification of c-*erb*B2 or c-*myc* has prognostic significance (Champeme *et al.*, 1994; Berns *et al.*, 1995; Deming *et al.*, 2000). In order to evaluate the clinical relevance of *FHIT* gene alterations in breast cancer, we analyzed the *FHIT* transcripts and the amplification of c-*erb*B2 and c-*myc*. To determine the gene copy number of the c-*erb*B2 and c-*myc* genes we used multiplex PCR. In the same tube two fragments of DNA were amplified, one representing the studied gene and the other a control gene. As a control gene for c-*myc* we chose the gene for tissue plasminogen activator (TPA). Both genes are located on chromosome 8. The gene for thymidine kinase (TK) located on 17q23.2 was the control gene for c-*erb*B2, placed on the same chromosome. Representative results of multiplex PCR are presented in Fig. 3. We found amplification of the c-*myc*

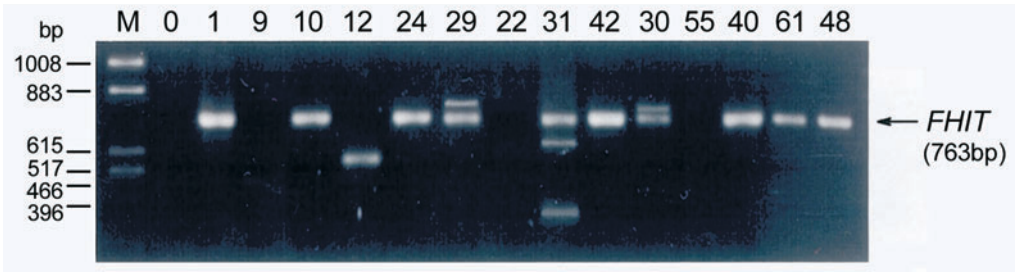

Figure 1. RT-PCR analysis of *FHIT* gene transcript in breast cancer tissues.

PCR products were separated on 2% agarose gel. Arrow indicates the normal-sized product of the *FHIT* gene (763 bp). Lane M, DNA molecular size markers; lane 0, product of control PCR performed without the reverse transcriptase product. Numbers above the lanes indicate the patient's number.

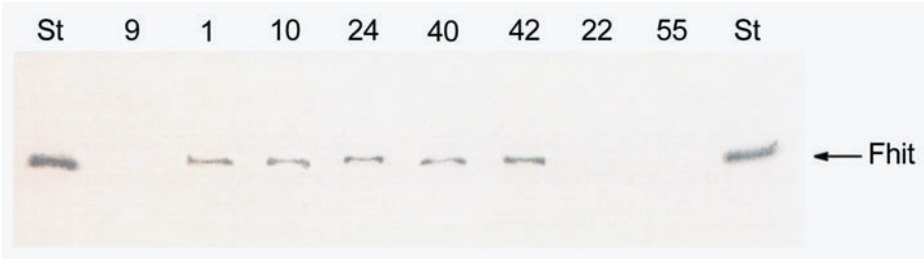

Figure 2. Immunoblot analysis of samples derived from breast cancer tissues.

Tissue extracts (100 ng of protein) were subjected to SDS/PAGE (12%) and transferred to an Immobilon transfer membrane. The membranes were immunobloted with anti-Fhit polyclonal antibodies. On lanes St recombinant Fhit protein (10 ng) was loaded. Numbers above the lanes indicate the patient's number. For *FHIT* transcript status in these tissues see Fig. 1.

Table 2. Relation of *FHIT* gene transcript status with histopathological type of tumor.

The presence of any abnormal transcript with or without accompanying normal-sized transcript was counted as "aberrant". *$P < 0.05$.

FHIT transcript	*ductale* n = 46	*lobulare* n = 25	*tubulare* n = 5	*mucinosum* n = 2	*medullare* n = 1
Normal	33	10	2	2	1
Aberrant	13	15*	3	–	–

gene in 16% of the tumors studied. Amplification of the c-*erb*B2 oncogene was observed in 21% of cancerous tissues. The percentage of c-*myc* and c-*erb*B2 amplification was of the same magnitude as reported by others in breast cancer.

Analyzing the occurrence of c-*myc* and c-*erb*B2 amplification and alteration of *FHIT* gene transcripts we found that normal *FHIT* transcript occurred more frequently (43 of 65) in cancers with the normal number of c-*myc* gene copies (Table 3). On the other hand, aberrant *FHIT* transcripts were found predominantly (64%) in cancers with c-*myc* amplification. Since the aberrant *FHIT* transcripts were frequently accompanied by c-*myc* amplification, we analyzed relation of the coexisting abnormalities to some clinical and epidemiological prognostic factors. We found that cancers having an aberrant *FHIT* gene transcript with accompanying c-*myc* amplification occurred more frequently ($P < 0.05$) in patients with positive lymph node status (Table 4).

DISCUSSION

In breast cancer as well as in carcinomas of the digestive tract in the most frequently observed aberrant *FHIT* transcripts exon 8 is missing (Ohta *et al.*, 1996; Negrini *et al.*, 1996). Exon 8 contains the histidine triad domain, which is essential for the anticancer activity of Fhit protein (Siprashvili *et al.*, 1997). Thus it is possible that in tumor cells the Fhit protein is inactivated by the appearance of *FHIT* gene transcripts lacking coding domain sequences essential for Fhit activity. However, we were unable to detect any abnormal-sized Fhit protein in tissues that displayed, besides the normal, also an aberrant *FHIT* transcript. This indicates that the abnormal transcripts do not encode any Fhit

Table 3. Relation of c-*myc* and c-*erb*B2 amplification with the status of *FHIT* gene transcript in cancerous tissues.

The presence of any abnormal transcript with or without accompanying normal-sized transcript was counted as "aberrant". *$P < 0.05$.

Status of *FHIT* gene transcript	Number of c-*myc* oncogene copies		Number of c-*erb*B2 oncogene copies	
	Normal n = 65	Amplification n = 14	Normal n = 61	Amplification n = 18
Normal	43*	5	40	8
Aberrant	22	9*	21	11

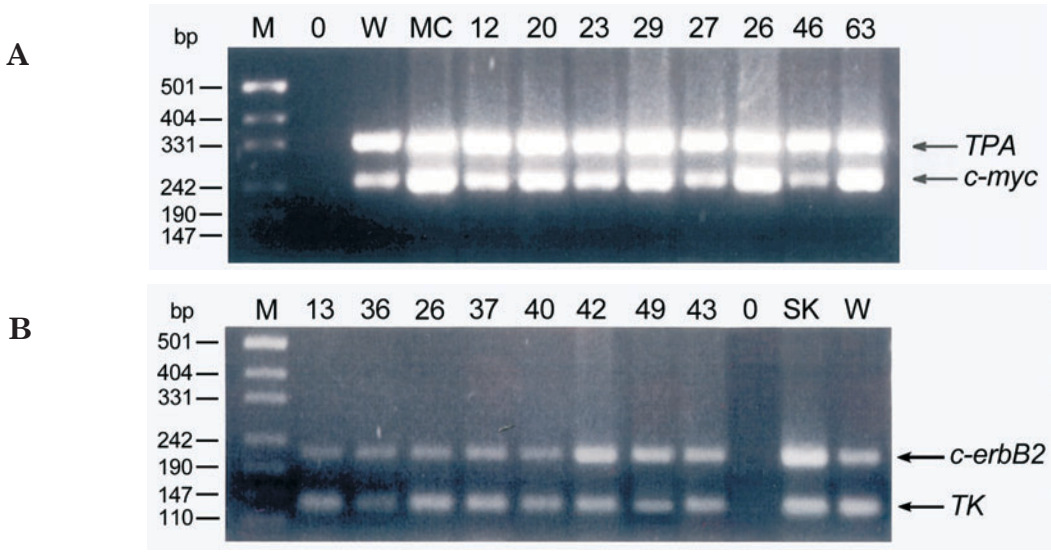

Figure 3. Analysis of c-*myc* and c-*erb*B2 amplification in breast cancer.

A: PCR analysis of c-*myc* amplification with the gene for tissue plasminogen activator (TPA) as a reference gene. **B**: PCR analysis of c-*erb*B2 amplification with the gene for thymidine kinase (TK) as a reference gene. Lane M, DNA molecular size markers; lane 0, product of control PCR performed without the template; lane W, PCR product with DNA isolated from a healthy person; lanes MC and SK, products of PCR performed with DNA isolated from cells with defined c-*myc* (MCF 7) and c-*erb*B2 (SKBR 3) amplification, respectively. Numbers above the lanes indicate the patient's number.

protein or the amount of such a protein is very low. The simultaneous presence of normal and aberrant *FHIT* transcripts within the same tumor cells has been reported (Negrini *et al.*, 1996; Guo *et al.*, 2000). However, data from studies on various cancer cell lines indicate that the cells in which normal and aberrant *FHIT* transcripts are present express only the normal-sized Fhit protein but not the truncated form (Druck *et al.*, 1997; Otterson *et al.*, 1998). On the other hand, the expression level of Fhit is reduced when this protein is translated from transcripts lacking the non-coding exons 3 or 4 (Sozzi *et al.*, 1997). In our studies we observed a reduced level of the Fhit protein in some cancers displaying both normal and aberrant *FHIT* transcripts and in cancers with the normal *FHIT* transcript only (not shown). Since primary cancers are polyclonal the interpretation of these observations is difficult. In several types of cancer a strong association of impaired Fhit protein expression with the disruption of *FHIT* transcript has been observed (Baffa *et al.*, 1998; Guo *et al.*, 2000; Huiping *et al.*, 2000; Yoshino *et al.*, 2000). It has been postulated that in some cancers, including breast cancer, alterations in the *FHIT* gene represent an early event in carcinogenesis (Baffa *et al.*, 1998; Zou *et al.*, 1999; Guo *et al.*, 2000; Huiping *et al.*, 2000), whereas in other cancers aberrant *FHIT* transcripts occur at later stages of cancer development (Yoshino *et al.*, 2000).

In order to assess the clinical relevance of *FHIT* transcript alteration in breast cancer, we have analyzed some clinical and epidemiological characteristics of patients having normal and aberrant *FHIT* transcripts. There was no association of *FHIT* transcript alteration with age, tumor size, estrogen and progesterone receptor status, local metastases and histological grading. Similar results have been reported by Hayashi *et al.* (1997) who analyzed a group of 61 Japanese patients. However, these investigators have found an association of the occurrence of aberrant *FHIT* transcripts with bilateral breast cancer and experience of childbirth. Since in our group of patients there were no bilateral breast cancer cases we could not make such an analysis. In

Table 4. Comparison of *c-myc* amplification with *FHIT* gene transcript status in breast cancer.

Relation with clinical and epidemiological characteristics of patients. *$P < 0.05$.

	c-myc amplification and normal *FHIT* n = 5	c-*myc* amplification and aberrant *FHIT* n = 9
Histological grading		
1	1	–
2	3	9
3	1	1
Lymph node status		
negative	4	2
positive	1	7*
Steroid receptors status		
ER(+)	3	3
ER(–)	2	6
PR(+)	2	4
PR(–)	3	5
Tumor size (mm)	20.4 ± 2.8	25.5 ± 7.9
Age (years)	62.1 ± 10.9	58.1 ± 13.1

our study we did not observe any correlation of *FHIT* transcript aberration with childbirth experience (not shown). On the other hand, we did observe a relation between the presence of aberrant *FHIT* transcripts and the histopathological type of tumor. The aberrant *FHIT* transcripts were more frequently observed in *lobular* cancers (60%) than in *ductal* cancers (28%), $P < 0.05$ (Table 2). Since *ductal* cancers are less differentiated than *lobular* cancers it could be assumed that abnormalities in *FHIT* mRNA depend on the stage of tumor cells differentiation. These tumors would be regarded as having a relatively good prognosis on the basis of conventional clinicopathological diagnosis, although *lobular* cancers have uncertain malignant potential.

Numerous studies on oncogene amplification in breast cancer have shown prognostic significance of c-*erb*B2 and c-*myc* amplification. The frequency of amplification of both oncogenes observed by us in the breast cancers examined was comparable to that reported by others (Borg *et al.*, 1992; Berns *et al.*, 1992; Chen *et al.*, 1995; Mark *et al.*, 1999; Deming *et al.*, 2000). We did not see any relations between c-*erb*B2 amplification and aberration of *FHIT* gene status. On the other hand, our study revealed a more frequent incidence of abnormal *FHIT* gene transcript occurrence in breast cancer displaying amplification of c-*myc*. The observed association of abnormalities in *FHIT* transcript with c-*myc* amplification would indicate that in breast cancer c-*myc* is somehow involved in inactivation of the *FHIT* gene. Since *FHIT* is regarded as a tumor suppressor gene, the observed association of aberrant *FHIT* gene transcript occurrence with c-*myc* amplification suggests that abnormal expression of c-*myc* may facilitate progression of breast neoplasia.

To date axillary lymph node status is the best prognostic indicator in breast cancer. Numerous clinical trials have established that involvement of axillary lymph nodes is related to high recurrence and early death rate. We found that aberrant *FHIT* transcript with accompanying c-*myc* amplification occurred more frequently in breast cancers with positive lymph node status (Table 4). This may indicate that alteration of *FHIT* transcript with accompanying c-*myc* amplification could have

a predictive value in breast cancer. However, to determine whether *FHIT* transcript aberration has a prognostic utility in breast cancer, extended clinical follow up will be necessary.

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REFERENCES

Ahmadian M, Wistuba II, Fong KM, Behrens C, Kodagoda DR, Saboorian MH, Shay J, Tomlinson GE, Blum J, Minna JD, Gazdar AF. (1997) Analysis of the *FHIT* gene and *FRA*3B region in sporadic breast cancer, preneoplastic lesions, and familial breast cancer probands. *Cancer Res.;* **57**: 3664–8.

Baffia R, Veronese ML, Santoro B, Mandes B, Palazzo JP, Rugge M, Santoro E, Croce CM, Huebner K. (1998) Laoo os *FIHT* expression in gastric carcinoma. *Cancer Res.*; **58**: 4708–14.

Barnes LD, Garrison PN, Siprashvili Z, Guranowski A, Robinson AK, Ingram SW, Croce CM, Ohta M, Huebner K. (1996) Fhit, a putative tumor suppressor in humans is a dinucleoside 5′,5′′-P^1,P^3-triphosphate hydrolase. *Biochemistry.;* **35**: 11529–35.

Berns EM, Klijn JG, van Staveren IL, Portengen H, Noordegraaf E, Foekens JA. (1992) Prevalence of amplification of the oncogenes c-*myc*, HER2/neu, and *int*-2 in one thousand human breast tumors: correlation with steroid receptors. *Eur J Cancer.;* **28**: 697–700.

Berns EM, Foekens JA, van Staveren IL, van Putten WL, de Koning HY, Portengen H, Klijn JG. (1995) Oncogene amplification and prognosis in breast cancer: relationship with systemic treatment. *Gene.;* **159**: 11–8.

Bloom HJ, Richardson WW. (1957) Histological grading and prognosis in breast cancer. *Br J Cancer.;* **11**: 359–77.

Borg A, Baldetrop B, Ferno M, Olsson H, Sigurdsson H. (1992) c-*myc* amplification is an independent prognostic factor in postmenopausal breast cancer. *Int J Cancer.;* **51**: 687–91.

Campiglio M, Pekarsky Y, Menard S, Tagliabue E, Pilotti S, Croce CM. (1999) FHIT loss of function in human primary breast cancer correlates with advanced stage of the disease. *Cancer Res.;* **59:** 3866–9.

Champeme MH, Bieche I, Hacene K, Lidereau R. (1994) Oncogene amplification *per se*: an independent prognostic factor in human breast cancer. *Mol Carcinog.;* **11**: 189–91.

Chen Y, Dong J, Lu Y, McGee JO. (1995) Quantitative detection of amplification of proto-oncogenes in breast cancer. *Chin Med J.;* **108**: 849–54.

Chrzan P, Skokowski J, Karmolinski A, Pawelczyk T. (2001) Amplification of c-*myc* gene and overexpression of c-Myc protein in breast cancer and adjacent non-neoplastic tissue. *Clin Biochem.;* **34**: 677–82.

Deming SL, Nass SJ, Dickson RB, Trock BJ. (2000) c-*myc* amplification in breast cancer: a meta-analysis of its occurrence and prognostic relevance. *Br J Cancer.;* **83**: 1688–95.

Druck T, Hadaczek P, Fu TB, Ohta M, Siprashvili Z, Baffa R, Negrini M, Kastury K, Veronese ML, Rosen D, Rothstein J, McCue P, Cotticelli MG, Inoue H, Croce CM, Huebner K. (1997) Structure and expression of the human *FHIT* gene in normal and tumor cells. *Cancer Res.;* **57**: 504–12.

Fong KM, Biesterveld EJ, Virmani A, Wistuba I, Sekido Y, Bader SA, Ahmadian M, Ong ST, Rassool FV, Zimmerman PV, Giaccone G, Gazdar AF, Minna JD. (1997) *FHIT* and *FRA*3B 3p14.2 allele loss are common in lung cancer and preneoplastic bronchial lesions and are associated with cancer-related *FHIT* cDNA splicing aberrations. *Cancer Res.;* **57**: 2256–67.

Guo Z, Johansson SL, Rhim JS, Vishwanatha JK. (2000) Fragile histidine triad gene expression in primary prostate cancer and in an *in vitro* model. *Prostate.;* **43**: 101–10.

Hayashi S, Tanimoto K, Hajiro-Nakanishi K, Tsuchiya E, Kurosumi M, Hagashi Y, Imai K, Suga K, Nakachi K. (1997) Abnormal *FHIT* transcripts in human breast carcinomas: a clinicopathological and epidemiological analysis of 61 Japanese cases. *Cancer Res.;* **5**: 1981–5.

Huiping C, Jonasson JG, Agnarsson BA, Sigbjornsdottir BI, Huebner K, Ingvarsson S. (2000) Analysis of the fragile histidine triad (*FHIT*) gene in lobular breast cancer. *Eur J Cancer.;* **36**: 1552–7.

Ingvarson S, Sigbjornsdottir BI, Huiping C, Jonasson JG, Agnarsson BA. (2001) Alterations of the *FHIT* gene in breast cancer: association with tumor progression and patient survival. *Cancer Detect Prev.;* **25:** 292–8.

Lonn U, Lonn S, Nilsson B, Stenkvist B. (1995) Prognostic value of *erb*-B2 and *myc* amplification in breast cancer imprints. *Cancer.;* **75**: 2681–7.

Mark HF, Aswad B, Bassily N, Taylor W, Brown S, Sun CL, Samy M, Zolnierz K, Wong E, Bland KI, Hsu PH. (1999) *HER-2/neu* gene amplification in stages I-IV breast cancer detected by fluorescent *in situ* hybridization. *Genet Med.;* **3**: 98–103.

Negrini M, Monaco C, Vorechovsky I, Ohta M, Druck T, Baffa R, Huebner K, Croce CM. (1996) The *FHIT* gene at 3p14.2 is abnormal in breast carcinomas. *Cancer Res.;* **56**: 3173–9.

Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM, Huebner K. (1996) 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**: 587–97.

Otterson GA, Xiao G-H, Geradts J, Jin F, Chen W, Niklinska W, Kaye FJ, Yeung RS. (1998) Protein expression and functional analysis of the *FHIT* gene in human tumor cells. *J Natl Cancer Inst.;* **90**: 426–32.

Pandis N, Bardi G, Mitelman F, Heim S. (1997) Deletion of the short arm of chromosome 3 in breast tumors. *Genes Chromosomes Cancer.;* **18**: 241–5.

Sakowicz M, Grden M, Pawelczyk T. (2001) Expression level of adenosine kinase in rat tissues. Lack of phosphate effect on the enzyme activity. *Acta Biochim Polon.;* **48**: 745–55.

Siprashvili Z, Sozzi G, Barnes LD, McCue P, Robinson AK, Eryomin V, Sard L, Tagliabue E, Greco A, Fusetti L, Schwartz G, Pierotti MA, Croce CM, Huebner K. (1997) Replacement of Fhit in cancer cells suppresses tumorigenicity. *Proc Natl Acad Sci U S A.;* **94**: 13771–6.

Sozzi G, Tornielli S, Tagliabue E, Sard L, Pezzella F, Pastorino U, Minolitti F, Pilotti S, Ratcliffe C, Veronese ML, Goldstraw P, Huebner K, Croce CM, Pierotti MA. (1997) Absence of Fhit protein in primary lung tumors and cells lines with *FHIT* gene abnormalities. *Cancer Res.;* **57**: 5207–12.

Watson PH, Safneck JR, Le K, Dubik D, Shiu RP. (1993) Relationship of c-*myc* amplification to progression of breast cancer from *in situ* to invasive tumor and lymph node metastasis. *J Natl Cancer Inst.;* **85**: 902–7.

Yoshino K, Enomoto T, Nakamura T, Sun H, Ozaki K, Nakashima R, Wanada H, Saitoh J, Watanabe Y, Noda K, Murata Y. (2000) *FHIT* alterations in cancerous and non-cancerous cervical epithelium. *Int J Cancer.*; **85**: 6–13.

Yuan BZ, Keck-Waggoner C, Zimonjic DB, Thorgeirsson SS, Popescu NC. (2000) Alterations of the *FHIT* gene in human hepatocellular carcinoma. *Cancer Res.;* **60:** 1049–53.

Zou TT, Lei J, Shi YQ, Yin J, Wang S, Souza RF, Kong D, Shimada Y, Smolinski KN, Greenwald BD, Abraham JM, Harpaz N, Meltzer SJ. (1997) *FHIT* gene alterations in esophageal cancer and ulcerative colitis (UC). *Oncogene.;* **15**: 101–15.

Zou M, Shi Y, Farid NR, al-Sedairy ST, Paterson MC. (1999) *FHIT* gene abnormalities in both benign and malignant thyroid tumors. *Eur J Cancer.;* **35**: 467–72.