Prevalence of Fragile Histidine Triad Expression in Tumors from Saudi Arabia: A Tissue Microarray Analysis

Prashant Bavi,[1] Zeenath Jehan,[2] Valerie Atizado,[1] Hassan Al-Dossari,[1] Fouad Al-Dayel,[3] Asmah Tulbah,[3] Samir S. Amr,[4] Salwa S. Sheikh,[4] Adnan Ezzat,[5] Hassan El-Solh,[6] Shahab Uddin,[2] and Khawla Al-Kuraya[1,2]

[1]Biological Repository Centre at Research Centre; [2]Research Centre at King Fahad National Center for Children's Cancer and Research, and [3]Department of Pathology, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia; [4]Pathology Services Division, Dhahran Health Centre, Saudi Aramco Medical Services Organization, Dhahran, Saudi Arabia; [5]Departments of Medical and Clinical Operations, and [6]Pediatric Oncology, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

Abstract

Aim: **The fragile histidine triad (*FHIT*) gene was discovered and proposed as a tumor suppressor gene for most human cancers. It encodes the most active common human chromosomal fragile region, FRA3B. We studied the prevalence of loss of FHIT expression in various tumors and correlated its loss with various clinicopathologic features.**

Methods: **To determine whether the absence of FHIT expression correlates with clinical variables such as grade, stage, and survival time, we assessed FHIT expression using immunohistochemistry. More than 1,800 tumors from more than 75 tumor categories were analyzed by immunohistochemistry in a tissue microarray format.**

Results: **Loss of FHIT expression ranged from 19% in ovarian tumors to 67% in lung cancers. Clinical and pathologic features like grade, stage, tumor size, and lymph node metastasis showed correlation with loss of FHIT expression in some tumors. No difference was seen in the survival patterns and loss of FHIT expression in any of the tumor groups studied.**

Conclusions: **Loss of FHIT expression is an ubiquitous event in the multistep, multifactorial carcinogenesis process. FHIT may be altered at different stages in different types of cancers. Most of the tumors with a wider prevalence of loss of FHIT expression as an early event show a correlation with clinicopathologic features. However, in some of the tumors, FHIT expression is lost as a late event and is only seen in a fraction of the tumors.** (Cancer Epidemiol Biomarkers Prev 2006;15(9):1708–18)

Introduction

Cancer is a genetic disease resulting from multiple, sequential genetic changes affecting oncogenes, tumor suppressor genes, and modifiers. Because of this multistep process, most human malignancies show various degrees of genetic heterogeneity even if they originate from single cells. Thus, cancer cells of the same clonal tumor mass may respond differently to chemotherapy or radiation therapy. Most of the human leukemias and lymphomas carry consistent chromosomal rearrangements, predominantly chromosomal translocations or inversions that activate specific oncogenes (1, 2) or cause loss of function of specific tumor suppressor genes (3), thereby initiating the process of malignant transformation. However, it is not known what the initiating events are for some of the most common human malignancies, the malignant epithelial tumors such as lung, breast, and prostate cancer.

Many tumor-suppressor genes, such as *rb, wt1, p53, nf1, apc, smad*, and *pten*, have been identified as tumor suppressor genes through loss of heterozygosity for polymorphic markers. Deletions in the short arm of chromosome 3 (3p loss of heterozygosity) are observed in various human cancers. At least three genes associated with the genesis of human cancer have been positioned on the short arm of chromosome 3p (4). Alterations of the von Hippel-Lindau (*vhl*) gene at 3p25 are frequently observed in renal cell carcinoma and pheochromocytoma (5). The *mlh1* gene, one of the mismatch repair genes which, when defective, cause hereditary non–polypoid colon carcinoma, exists at 3p21.3 (6). The telomerase repressor gene, which suppresses the expression of the telomerase gene, also exists on chromosome 3p (7).

The fragile histidine triad (*FHIT*) gene is a candidate tumor suppressor gene located at chromosome 3p14.2, spanning the FRA3B common fragile site (8). The FHIT protein is homologous to Ap4A hydrolase from the yeast, *Schizosaccharomyces pombe*, and it also exhibits Ap3A activity in enzymatic assays (8, 9). FHIT protein is presumed to have tumor suppressor function independent of its hydrolase activity (8-10). However, the mechanisms through which FHIT mediates its suppressor function are not well established. Several investigators have shown that introduction of a wild-type *FHIT* gene suppresses tumorigenicity, and the transfection of FHIT in FHIT-deficient human cancer cells seem to induce apoptosis and inhibit cell growth (10-12). These results suggest that the suppressor activity of FHIT could be related with apoptosis and with the alteration of cell cycle regulator factors. Frequent allelic losses and homozygous deletions (8, 9, 13-24), as well as the loss of heterozygosity in microsatellites located at the *FHIT* gene have been described at the *FHIT* locus in several human solid tumors arising from epithelial cells (13, 19, 20, 25-32). Studies comparing FHIT expression with the status of the *FHIT* gene have shown significant concordance in a variety of malignancies. Abnormalities in the *FHIT* gene and/or its expression have been identified in a variety of human cancer cell lines and tumor tissues including lung (33), breast (34-36), urinary bladder (37), head and neck (38), esophageal (39, 40), gastric (41), colorectal (42), renal (43), liver cancers (44), and diffuse large B cell lymphoma (45).

To determine the role of FHIT in the pathogenesis and progression of cancer, we investigated the incidence of loss of

Received 12/26/05; revised 4/23/06; accepted 7/10/06.

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.

Requests for reprints: Khawla Al-Kuraya, Research Centre at KFNCCC&R, King Faisal Specialist Hospital and Research Centre, MBC 98-16, P.O. Box 3354, Riyadh 11211, Saudi Arabia. Phone: 966-1-229-4444, ext. 51813; Fax: 966-1-205-5170. E-mail: kkuraya@kfshrc.edu.sa

doi:10.1158/1055-9965.EPI-05-0972

FHIT expression by immunohistochemistry in a series of tumors in a tissue microarray format. We also correlated FHIT expression with age, sex, histology diagnosis, grade, and stage of various tumors.

Materials and Methods

A total of 1,889 specimens from the archives of the King Faisal Specialist Hospital and Research Centre (Riyadh, Saudi Arabia) were assessed, including tumors from 15 different sites and 75 tumors and subtypes. A multitumor array block comprised of 578 specimens from 13 different sites and 9 other array blocks were constructed from tumors of the breast, colon, liver, lung, lymphomas, kidney, and meningiomas (Table 1).

Tissue Microarray. TMA construction was done as described previously (46). Briefly, all the H&E slides were screened and nonnecrotic well-fixed tumor areas were mapped with an indelible marker pen. Using these mapped slides as a reference, 0.6 mm diameter punches were obtained from the donor blocks. The tissue microarrayer (Beecher Instruments, Woodland, WI) was used. A map of the recipient block was prepared with coordinates and a number for each sample to correctly identify the tumors. The punched-out tissue cores from the donor block were inserted in the recipient block. The cores were arranged 8 mm from the edges and the distance between two cores was 0.8 mm. The array blocks were incubated at 45°C for 10 minutes to improve adhesion between cores and paraffin of the recipient block. They were cut at room temperature with a standard microtome (Thermo Shandon, Cheshire, United Kingdom) and slides were prepared using tape sectioning system (Instrumedics, Inc., St. Louis, MO).

Immunohistochemistry. Sections of 5 μm from all the array blocks were cut, slides were deparaffinized in xylene and rehydrated in pure ethanol. Endogenous peroxidase was blocked using 3% hydrogen peroxidase in methanol for 10 minutes. Antigen retrieval was done by placing the sides in a citrate buffer (pH 6.0) and microwaving them for 5 minutes at 750 W and for 15 minutes at 250 W.

The sections were incubated for 90 minutes in 1:900 dilutions of polyclonal rabbit antibodies reacting against FHIT protein (ZR44; Zymed, San Francisco, CA). Bound antibody was detected with biotinylated link antibody (Dako, Carpinteria, CA) and horseradish peroxidase–labeled streptavidin (Dako). Color was developed in 3,3′-diaminobenzidine with H_2O_2 as substrate (Dako). The sections were then washed in running tap water, lightly counterstained with Gill's hematoxylin, dehydrated through ascending graded alcohols, cleared in xylene, and mounted in DPX. All normal epithelia (lung, liver, urinary bladder, and colon) showed strong cytoplasmic expression of FHIT and served as an internal positive control. Separate negative controls (renal glomeruli and lung cancer) were appropriately negative for FHIT protein. In addition, no staining was observed when primary antibody was replaced by normal rabbit serum IgG. Expression was scored on a three-tiered scale for both intensity (grade 1, absent/weak; grade 2, moderate; grade 3, strong) and extent (grade 1, percentage of positive cells is <10%; grade 2, 10-50%; grade 3, >50%). The intensity and extent scores were multiplied to give a composite score (1-9) for each tumor. A score of 0 was defined as absent or lost expression, scores of 1 to 3 were defined as markedly reduced FHIT expression, and scores of 4 to 9 were considered as normal expression (47-49). Because at least three different slides for each array constructed was stained with anti-FHIT antibody, the mean of all three FHIT scores was used for statistical analyses.

Statistical Analysis. Statistical analysis was done using SAS version JMP IN 5.1 software (SAS Institute, Cary, NC), and all P values reported are two-tailed. Univariate analysis of categorical variables was conducted using contingency analysis and χ^2 tests. The surviving fraction was estimated using the Kaplan-Meier method. In the final model, all variables were considered statistically significant at $P < 0.05$. Univariate analysis of FHIT expression was done with age, sex, grade, stage, and survival in breast, colon, kidney, lung, liver cancers, and diffuse large B cell lymphomas.

Results

In normal tissues, a uniform strong cytoplasmic staining of FHIT was seen in all epithelial cells of the skin, breast, colon, liver, lung, kidney, ovary, esophagus, urinary bladder, and stomach. Similar FHIT expression was seen in normal lymphoid cells as well as in the soft tissues.

FHIT expression was found to be reduced by 19% to 100% in the various tumor types studied (Table 2). Decreased or absent FHIT expression was seen in 46.3% of the breast carcinomas. No correlation was seen between FHIT expression and age, sex, histology diagnosis (H&E diagnosis), and survival pattern. Loss of FHIT expression was correlated with higher grade ($P = 0.0187$) and advanced stage ($P = 0.030$; Table 3). Decreased or absent FHIT expression was seen in 20.7% of the colorectal adenocarcinomas and a positive correlation was observed between loss of FHIT expression and advanced stage (Dukes) of colorectal cancers ($P = 0.0392$). No correlation was seen between FHIT expression and age, sex, H&E diagnosis, grade, and survival pattern (Table 4).

Table 1. TMA blocks and sample distribution

Serial no.	System	No. of array blocks	Total no. of specimens	Total no. of FHIT analyzed	No. of tissue/tumors	Discards/repeats
1	Bladder	1 (MTA)	69	48	21	0
2	Breast	2+ (MTA)	651	467	168	16
3	Colon	2+ (MTA)	295	227	22	46
4	Esophagus	1 (MTA)	6	4	2	0
5	Head and neck	1 (MTA)	33	17	16	0
6	Kidney	1+ (MTA)	158	144	13	1
7	Liver	1+ (MTA)	111	100	8	3
8	Lung	1	68	62	5	1
9	Lymphoma	1+ (MTA)	216	149	34	33
10	Meningioma	1	97	88	9	0
11	Nasopharynx	1 (MTA)	11	9	2	0
12	Ovary	1 (MTA)	25	21	4	0
13	Skin	1 (MTA)	64	47	17	0
14	Soft tissue	1 (MTA)	40	35	5	0
15	Stomach	1 (MTA)	45	34	11	0
		10	1,889	1,452	337	100

Abbreviation: MTA, multitumor array block.

Table 2. Results of FHIT staining in various tumors

System	No.	Absent (%)	Reduced (%)	Normal (%)
Breast	467	26 (5.6)	191 (40.9)	250 (53.5)
Infiltrating duct carcinoma	446	23 (5.2)	186 (41.7)	237 (53.1)
Infiltrating lobular carcinoma	10	1 (10.0)	0 (0.0)	9 (90.0)
Malignant phylloid	4	1 (25.0)	3 (75.0)	0 (0.0)
Medullary carcinoma	3	1 (33.3)	1 (33.3)	1 (33.3)
Metaplastic carcinoma	1	0 (0.0)	0 (0.0)	1 (100.0)
Mucinous carcinoma	3	0 (0.0)	1 (33.3)	2 (66.7)
Colon	227	5 (2.2)	42 (18.5)	180 (79.3)
Adenocarcinoma	221	5 (2.3)	40 (18.1)	176 (79.6)
Mucinous carcinoma	6	0 (0.0)	2 (33.3)	4 (66.7)
Esophagus	4	2 (50.0)	2 (50.0)	0 (0.0)
Adenocarcinoma	1	0 (0.0)	1 (100.0)	0 (0.0)
Squamous cell carcinoma	3	2 (66.7)	1 (33.3)	0 (0.0)
Head and neck	17	0 (0.0)	11 (64.7)	6 (35.3)
Ca ex-pleomorphic adenoma	1	0 (0.0)	0 (0.0)	1 (100.0)
Mucoepidermoid carcinoma	1	0 (0.0)	1 (100.0)	0 (0.0)
Plasmacytoma	1	0 (0.0)	0 (0.0)	1 (100.0)
Squamous cell carcinoma	14	0 (0.0)	10 (71.4)	4 (28.6)
Kidney	144	3 (2.1)	42 (29.2)	99 (68.8)
Chromophobe cell carcinoma	18	0 (0.0)	1 (5.6)	17 (94.4)
Collecting duct carcinoma	1	0 (0.0)	0 (0.0)	1 (100.0)
Multilobular cystic renal carcinoma	2	0 (0.0)	1 (50.0)	1 (50.0)
Nephroblastoma/Wilms tumor	3	1 (33.3)	1 (33.3)	1 (33.3)
Papillary chromophil carcinoma	10	0 (0.0)	2 (20.0)	8 (80.0)
Renal cell carcinoma	101	2 (2.0)	31 (30.7)	68 (67.3)
Squamous cell carcinoma	1	0 (0.0)	1 (100.0)	0 (0.0)
Transitional cell carcinoma	8	0 (0.0)	5 (62.5)	3 (37.5)
Lymphoma	149	15 (10.1)	83 (55.7)	51 (34.2)
Anaplastic large cell lymphoma	2	0 (0.0)	0 (0.0)	2 (100.0)
Diffuse large B cell lymphoma	147	15 (10.2)	83 (56.5)	49 (33.3)
Lung	62	12 (19.4)	30 (48.4)	20 (32.3)
Adenocarcinoma	25	2 (8.0)	13 (52.0)	10 (40.0)
Bronchioalveolar carcinoma	4	0 (0.0)	2 (50.0)	2 (50.0)
Carcinoid tumor	7	0 (0.0)	1 (14.3)	6 (85.7)
Large cell carcinoma	3	2 (66.7)	0 (0.0)	1 (33.3)
Small cell carcinoma	8	4 (50.0)	4 (50.0)	0 (0.0)
Squamous cell carcinoma	15	4 (26.7)	10 (66.7)	1 (6.7)
Liver	100	9 (9.0)	36 (36.0)	55 (55.0)
Biliary mucinous cyst adenocarcinoma	1	0 (0.0)	1 (100.0)	0 (0.0)
Cholangiocarcinoma	5	2 (40.0)	2 (40.0)	1 (20.0)
Hepatocellular carcinoma	92	7 (7.6)	32 (34.8)	53 (57.6)
Neuroendocrine carcinoma	2	0 (0.0)	1 (50.0)	1 (50.0)
Meningioma	88	3 (3.4)	21 (23.9)	64 (72.7)
Angiomatous	2	0 (0.0)	0 (0.0)	2 (100.0)
Chordoid	1	0 (0.0)	0 (0.0)	1 (100.0)
Fibroblastic	15	2 (13.3)	4 (26.7)	9 (60.0)
Meningothelial	20	0 (0.0)	4 (20.0)	16 (80.0)
Secretory	1	0 (0.0)	0 (0.0)	1 (100.0)
Transitional	49	1 (2.0)	13 (26.5)	35 (71.4)
Nasopharynx	9	1 (11.1)	8 (88.9)	0 (0.0)
Nasopharyngeal type carcinoma	6	0 (0.0)	6 (100.0)	0 (0.0)
Squamous cell carcinoma	3	1 (33.3)	2 (66.7)	0 (0.0)
Ovary	21	2 (9.5)	2 (9.5)	17 (81.0)
Adenocarcinoma, NOS	2	0 (0.0)	0 (0.0)	2 (100.0)
Dysgerminoma	1	0 (0.0)	0 (0.0)	1 (100.0)
Endodermal sinus tumor	2	1 (50.0)	1 (50.0)	0 (0.0)
Endometrioid carcinoma	1	0 (0.0)	1 (100.0)	0 (0.0)
Granulosa cell tumor	1	0 (0.0)	0 (0.0)	1 (100.0)
Mucinous cystadenoma, borderline malignancy	1	0 (0.0)	0 (0.0)	1 (100.0)
Papillary serous cystadenocarcinoma	12	0 (0.0)	0 (0.0)	12 (100.0)
Papillary serous cystadenocarcinoma, borderline malignancy	1	1 (100.0)	0 (0.0)	0 (0.0)
Skin	47	0 (0.0)	34 (72.3)	13 (27.7)
Basal cell carcinoma	30	0 (0.0)	22 (73.3)	8 (26.7)
Kaposiform hemangioendothelioma	3	0 (0.0)	3 (100.0)	0 (0.0)
Keratoacanthoma	1	0 (0.0)	1 (100.0)	0 (0.0)
Melanoma	2	0 (0.0)	0 (0.0)	2 (100.0)
Squamous cell carcinoma	10	0 (0.0)	7 (70.0)	3 (30.0)
Verrucous carcinoma	1	0 (0.0)	1 (100.0)	0 (0.0)
Soft tissue tumor	35	15 (42.9)	13 (37.1)	7 (20.0)
Epithelioid sarcoma	2	1 (50.0)	1 (50.0)	0 (0.0)
Fibromyosarcoma	2	2 (100.0)	0 (0.0)	0 (0.0)
Leiomyosarcoma	2	0 (0.0)	0 (0.0)	2 (100.0)
Liposarcoma	3	0 (0.0)	2 (66.7)	1 (33.3)
Malignant peripheral nerve sheath tumor	2	0 (0.0)	1 (50.0)	1 (50.0)
Paraganglioma	1	0 (0.0)	0 (0.0)	1 (100.0)
Pleomorphic sarcoma	2	1 (50.0)	1 (50.0)	0 (0.0)

(*Continued on the following page*)

Table 2. Results of FHIT staining in various tumors (Cont'd)

System	No.	Absent (%)	Reduced (%)	Normal (%)
Primitive neuroectodermal tumor	2	0 (0.0)	0 (0.0)	2 (100.0)
Dermatofibrosarcoma protuberans	17	10 (58.8)	7 (41.2)	0 (0.0)
Rhabdomyosarcoma	2	1 (50.0)	1 (50.0)	0 (0.0)
Stomach	34	0 (0.0)	8 (24.0)	26 (76.0)
Adenocarcinoma	26	0 (0.0)	7 (26.9)	19 (73.1)
Carcinoid of stomach	1	0 (0.0)	1 (100.0)	0 (0.0)
Mucinous carcinoma	1	0 (0.0)	0 (0.0)	1 (100.0)
Signet cell ring carcinoma	6	0 (0.0)	0 (0.0)	6 (100.0)
Urinary bladder	48	0 (0.0)	26 (54.0)	22 (46.0)
Benign urothelial papilloma	5	0 (0.0)	4 (80.0)	1 (20.0)
Invasive urothelial carcinoma—high grade	4	0 (0.0)	3 (75.0)	1 (25.0)
Papillary urothelial carcinoma—high grade	11	0 (0.0)	5 (45.5)	6 (54.5)
Papillary urothelial carcinoma—low grade	23	0 (0.0)	11 (47.8)	12 (52.2)
Papillary urothelial neoplasm—low malignant potential	3	0 (0.0)	1 (33.3)	2 (66.7)
Squamous cell carcinoma	2	0 (0.0)	2 (100.0)	0 (0.0)

Renal cancers showed absent or reduced FHIT expression in 31% of the tumors, and there was a statistically significant inverse correlation between loss of FHIT expression and grade ($P = 0.006$) and stage ($P = 0.023$). No correlation was seen between FHIT expression and age, sex, H&E diagnosis, and survival pattern (Table 5). Decreased or absent FHIT expression was seen in 45% of the liver carcinomas and a positive correlation was observed between loss of FHIT expression and higher grade and lymph node metastasis. No correlation was seen between FHIT expression and age, sex, H&E diagnosis, and survival pattern (Table 6). FHIT expression was reduced or absent in 67.8% of the lung tumors. Tumors showing loss of FHIT expression were of a higher stage ($P = 0.031$), larger size ($P = 0.046$), and occurred more commonly in the older age group ($P = 0.006$). FHIT expression was also correlated with histology ($P = 0.003$). One hundred percent of the small cell carcinomas and 92% of squamous cell carcinomas showed a reduced or absent FHIT expression as compared with 54% of adenocarcinomas or 25% of bronchioalveolar carcinomas (Table 7).

Table 3. Correlation of clinicopathologic features with FHIT staining in breast carcinomas

Clinicopathologic features	No.	FHIT Results			*P*
		Normal	Reduced	Absent	
Age					Not significant
0-40	222	50	33	05	0.8998
41-60		60	38	09	
>60		17	08	02	
H&E					Not significant
Infiltrating duct	199	106	75	12	0.1667
Carcinoma		03	00	00	
Lobular carcinoma		03	00	00	
Others					
Grade					0.0187
Grade 1	197	01	00	01	
Grade 2		41	15	05	
Grade 3		68	60	06	
Stage					0.0307
I	206	04	00	01	
II		62	38	05	
III		36	29	03	
IV		15	07	06	
Size (cm)					Not significant
<5	204	78	45	10	0.5753
5-10		32	25	04	
>10		04	04	02	
Lymph nodes positive					Not significant
Negative	191	13	13	03	0.3622
Positive		95	57	10	
Survival analysis					Not significant 0.1250

Loss of FHIT expression was seen in 65.78% of the non–Hodgkin's diffuse large B cell lymphomas. No correlation was seen between FHIT expression and age, sex, stage, and survival patterns (Table 8).

Reduced or absent FHIT expression was seen in 64.7% of head and neck cancers, 19% of ovarian tumors, 23.5% of gastric tumors, 54% of urinary bladder tumors, 72.3% of skin tumors, 27.3% of the meningiomas, and 80% of soft tissue tumors. Reduced or absent FHIT expression was seen in 100% of the nasopharyngeal (only nine cases) as well as in all four cases of esophageal carcinomas (Fig. 1).

Discussion

Chromosomal abnormalities, including homozygous deletions and loss of heterozygosity, are among the most common features of human tumors. The short arm of human chromosome 3, particularly the 3p14.2 region, is a major site of such rearrangements. The 3p14.2 region spans the most active common fragile site of the human genome (4), encompassing a familial kidney cancer–associated breakpoint (5), mismatch repair gene (6), and a telomerase repressor gene site (7).

The *FHIT* gene is a candidate tumor suppressor gene that was identified in this region by positional cloning (8). FHIT encompasses FRA 3B, the most common fragile site in the human genome (8). Subsequent studies have shown that *FHIT* is commonly the target of chromosomal aberrations involving the short arm of human chromosome 3 and is thereby inactivated in most of the common human malignant diseases, including cancers of the lung (13), esophagus (39, 40), stomach (41), breast (17, 18, 34-36), and kidney (22).

The *FHIT* gene and its protein product have been the focus of recent debate with regard to their potential role in tumorigenesis (50). A tumor suppressor role for FHIT has been postulated based on the ability of FHIT to eliminate or reduce the tumorigenicity of tumor cells in nude mice (51). Loss of gene expression associated with morphologic progression from normal, nonneoplastic epithelium, through stages of hyperplasia and carcinoma *in situ* to invasive carcinoma, has generally been accepted as evidence of the suppressor role of that gene (52, 53).

The way(s) in which FHIT functions as a tumor suppressor gene is/are unknown, but FHIT protein has a proapoptotic effect when restored to FHIT protein–deficient cell lines (54). The FHIT protein is a dinucleoside 50, 5000-P1, P3-triphosphate (Ap3A) hydrolase (55) that produces ADP and AMP, although the tumor suppressor effect seems to be more strongly linked to substrate binding than substrate hydrolysis (56). FHIT mRNA and protein expression is found in most human tissues and genetic alterations are found in many

Table 4. Correlation of clinicopathologic features with FHIT staining in colorectal carcinomas

Clinicopathologic features	No.	FHIT Results			*P*
		Normal	Reduced	Absent	
Age	171				Not significant
0-40		21	07	02	0.6115
41-60		68	17	02	
>60		45	08	01	
Sex	171				Not significant
Female		65	17	03	0.8025
Male		69	15	02	
H&E	163				Not significant
Adenocarcinoma		123	30	05	0.5037
Mucinous carcinoma		03	02	00	
Grade	159				Not significant
Grade 1		01	01	00	0.8062
Grade 2		114	28	04	
Grade 3		09	02	00	
Stage	161				0.0355
I		21	07	00	
II		49	06	01	
III		47	08	02	
IV		11	09	00	
Size (cm)	137				Not significant
<5		56	10	03	0.3784
5-10		52	10	00	
>10		05	01	00	
Lymph nodes positive	155				Not significant
Negative		67	15	02	0.8574
Positive		54	15	02	
Survival analysis					Not significant 0.7858

human carcinomas, including loss of heterozygosity and translocations. Point mutations seem to be less common. FHIT mRNA splice variants are common in carcinoma, but are also frequently found in nonneoplastic tissues from healthy individuals (57). The recent findings in the molecular biology of FHIT, with particular focus on the opportunities for treatment and prevention of cancer, have been previously described (58).

Table 5. Correlation of clinicopathologic features with FHIT staining in renal clear cell carcinomas

Clinicopathologic features	No.	FHIT Results			*P*
		Normal	Reduced	Absent	
Age	110				Not significant
0-40		15	05	01	0.6614
41-60		39	17	02	
>60		23	08	00	
Sex	110				Not significant
Female		31	11	02	0.6034
Male		46	19	01	
H&E	107				Not significant
Chromophobe cell carcinoma		12	01	00	0.1188
Collecting duct carcinoma		01	00	00	
Multilocular cystic renal carcinoma		01	01	00	
Papillary chromophil carcinoma		06	00	00	
Renal cell carcinoma		54	22	02	
Transitional cell carcinoma		02	05	00	
Grade	109				0.0067
Grade 1		04	06	00	
Grade 2		51	17	01	
Grade 3		20	05	00	
Grade 4		01	02	02	
Stage	108				0.0234
I		37	16	00	
II		19	03	01	
III		15	04	00	
IV		05	06	02	
Size (cm)	104				Not significant
<5		21	13	00	0.1902
5-10		34	12	00	
>10		19	04	01	
Lymph nodes positive	17				Not significant
Negative		08	01	00	0.9294
Positive		07	01	00	
Survival analysis					Not significant 0.2676

Table 6. Correlation of clinicopathologic features with FHIT staining in liver carcinomas

Clinicopathologic features	No.	FHIT Results			*P*
		Normal	Reduced	Absent	
Age	87				Not significant
0-40		12	07	00	0.2877
41-60		17	10	04	
>60		22	10	05	
Sex	87				Not significant
Female		21	10	03	0.8743
Male		30	17	06	
H&E	68				Not significant
Cholangiocarcinoma		01	02	02	0.1780
Hepatocellular carcinoma		38	16	05	
Others		02	02	00	
Grade	64				0.0274
Grade 1		11	02	01	
Grade 2		26	10	06	
Grade 3		02	06	00	
Stage	56				Not significant
I		00	00	00	0.1156
II		16	03	00	
III		16	05	03	
IV		08	05	00	
Size (cm)	60				Not significant
<5		12	03	01	0.8117
5-10		20	05	01	
>10		11	06	01	
Lymph nodes positive	21				0.0392
Negative		15	03	01	
Positive		00	02	00	
Survival analysis					Not significant 0.4921

In an elegant and concise review, the types of cancers in which FHIT is associated with specific clinical features and the importance of further investigation of the consequences of FHIT loss in these cancers has been highlighted (59). The treatment of oral and esophageal cancers could be disfiguring and debilitating, but these sites are highly accessible to topical treatment using gene therapy approaches to prevention and might be candidates for future clinical trials using

Table 7. Correlation of clinicopathologic features with FHIT staining in lung carcinomas

Clinicopathologic features	No.	FHIT Results			*P*
		Normal	Reduced	Absent	
Age	66				0.0061
0-40		08	03	00	
41-60		04	17	05	
>60		10	12	07	
Sex	66				Not significant
Female		08	08	02	0.4271
Male		14	24	10	
H&E	62				0.0003
Adenocarcinoma		10	13	02	
Bronchioalveolar carcinoma		02	02	00	
Carcinoid tumor		06	01	00	
Large cell carcinoma		01	00	02	
Small cell carcinoma		00	04	04	
Squamous cell carcinoma		01	10	04	
Grade	40				Not significant
Grade 1		03	04	00	0.6963
Grade 2		08	14	04	
Grade 3		02	04	01	
Stage		51	0.0312		
I		10	11	00	
II		01	06	02	
III		03	07	05	
IV		01	04	01	
Size (cm)	50				0.0463
<5		14	13	02	
5-10		03	12	04	
>10		01	00	01	
Lymph nodes positive	41				Not significant
Negative		11	15	01	0.0617
Positive		03	07	04	
Survival analysis					Not significant 0.4934

Table 8. Correlation of clinicopathologic features with FHIT staining in diffuse large B cell lymphomas

Clinicopathologic features	No.	FHIT Results			*P*
		Normal	Reduced	Absent	
Stage	72				Not significant
I		05	13	01	0.8618
II		09	27	04	
III		01	04	01	
IV		03	03	01	
Survival analysis					Not significant 0.2962

viral FHIT delivery (59). Recently, viral *FHIT* gene transfer successfully prevented and reversed carcinogen-induced epithelial tumor formation in the forestomachs of FHIT-deficient mice (60, 61).

Ishii et al. have lucidly illustrated and explained that FHIT may be altered at different levels in different types of cancer (62). In the same article, they have hypothesized a schema, illustrating tumor cell propagation from possible ancestral cancerous or tumor stem cells with self-renewal potential to daughter cells, in which FHIT may be altered at different stages of precancer. They divided tumors into three classes:

Class A: In some tumors, FHIT is inactivated in a precursor cell, which results in loss of tumor suppressor function and leads to the expansion of tumor cells. In tumors of this category, aberration of the *FHIT* gene and FHIT protein reduction are associated with increased tumor proliferation, decreased apoptosis, and poorer survival of patients, as shown in lung and head and neck cancers (23, 26-28). The restoration of FHIT expression would be effective in the regression of such tumors, which are thus candidates for *FHIT* gene therapy, as supported by *in vitro* studies and animal experiments (60, 61).

Class B: In tumors in which the biological behavior seems to be unrelated to FHIT status, other factors could drive tumor progression. Several studies showed that although FHIT is altered in an early stage of cervical carcinogenesis, association of FHIT loss with histopathologic grades or clinicopathologic variables is not necessarily observed (63, 64). This may be because other factors, such as human papilloma virus infection in cervical cancer, drive tumor progression.

Class C: In some hematopoietic disorders, such as chronic myelogenous leukemia, tumor stem cells may exist far upstream from the FHIT-negative cells, as shown in case C. A relatively large study with a total of 195 Philadelphia chromosome–positive chronic myelogenous leukemias showed that lack of FHIT protein expression was detected in 4% of cases, and reduced FHIT expression was not associated with progression, response to therapy, or with prognosis in chronic myelogenous leukemia (65).

The TMA approach is optimally suited to identify those samples with frequent alterations of a specific gene (66, 67). They serve as an excellent tool to develop and compare immunohistochemical analysis. Hundreds of tumors can be immunostained under standardized conditions on one TMA slide. The small diameter of each arrayed tissue sample limits the comparison to a very small tissue area with a minimal likelihood of genetic, tissue processing, or immunostaining heterogeneity. Although the total number of abnormal cases detected by TMA may be low due to regional heterogeneity of immunostaining, the perfect standardization of staining more than compensates for it.

In our study, we analyzed FHIT by immunohistochemistry on three TMA blocks of breast, colon, lung, liver, and kidney tumors. The mean of the three FHIT scores obtained was taken as the final FHIT score for that specimen. Those specimens showing heterogeneous staining in the three array slides were reviewed again. Those specimens which showed one TMA slide with normal FHIT staining (score >3) and the other showing reduced (score <3) or absent (score = 0) were labeled as heterogeneous or discordant staining. These constituted a very small percentage (0-12.5%).

Reduced or absent FHIT expression was seen in only 46.5% of the breast cancers studied. Around 10% of the cases showed heterogeneous immunohistochemistry staining in the three TMA slides and were indicative of the incidence of

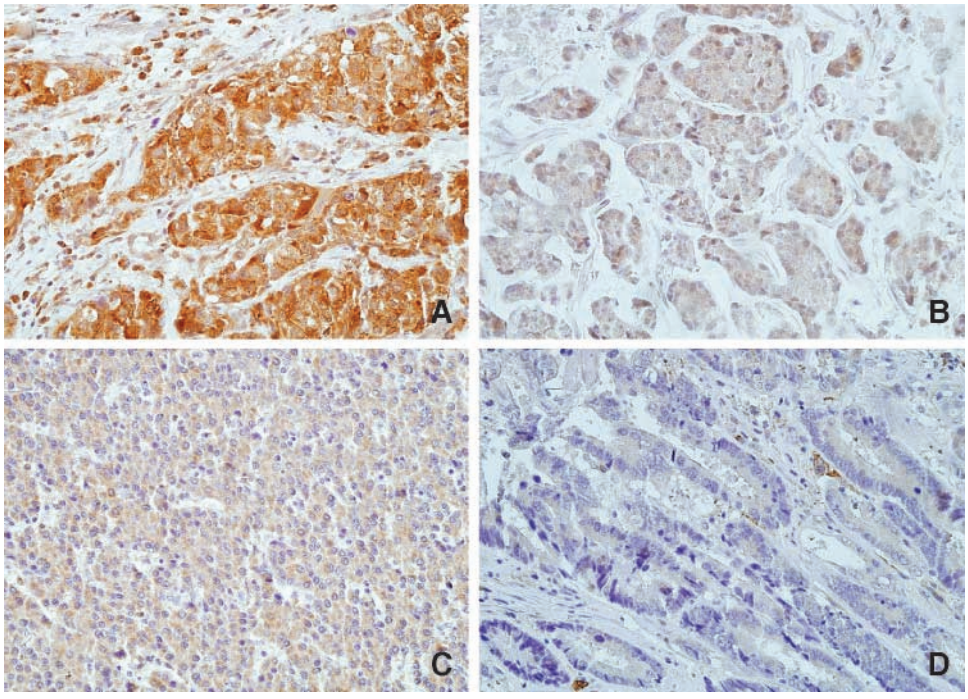

Figure 1. FHIT expression in various tumors (magnification, ×40). **A,** breast carcinoma with normal FHIT expression (score 9); **B,** breast carcinoma with reduced FHIT expression (score 3); **C,** liver carcinoma with reduced FHIT expression (score 3); **D,** colorectal adenocarcinoma with absent FHIT expression (score 0).

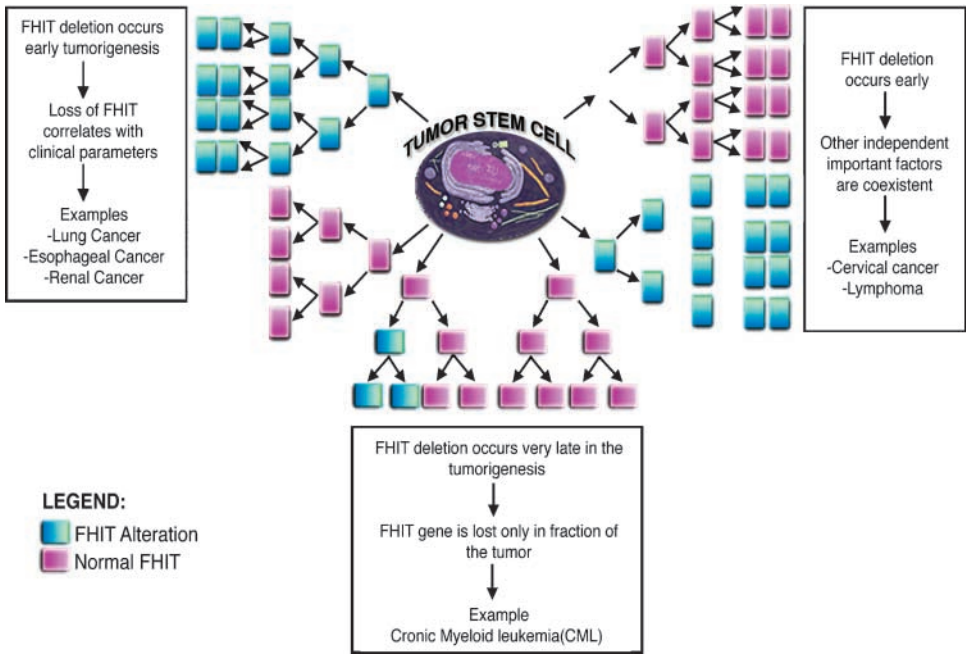

Figure 2. Model of FHIT inactivation in tumorigenesis of different types of cancer.

heterogeneous FHIT staining. This is in contrast to earlier studies which have found reduced FHIT expression in 69% to 76% of the breast cancers (34-36). However, a study done in a Japanese population showed that the loss of FHIT expression was seen in 42.2% of the cases studied (68). FHIT alteration may not be an early event in breast carcinogenesis in Saudi Arabia, as the prevalence of loss of FHIT expression is much lower. Earlier and ongoing studies have found that the behavior of breast carcinomas from Saudi Arabia is different compared with Western countries (69). Further studies involving the analysis of the *FHIT* gene to observe allelic loss and abnormal transcripts are needed and are being done in our set-up to study and confirm these differences.

FHIT expression was reduced or absent in only 20.7% of the colorectal carcinomas. An earlier study showed loss or reduced FHIT expression in 44% of the colorectal cancers (42). No correlation was seen between FHIT expression and age, sex, H&E diagnosis, grade, tumor size, lymph node metastasis, and survival time. A positive correlation was observed between loss of FHIT expression and advanced stage (Dukes) of colorectal cancers ($P = 0.0392$). Loss of FHIT expression was seen in the earlier studies in the range of 23% to 50% using immunohistochemistry (70-72). Loss of FHIT expression was correlated with higher grade, Dukes stage, distant metastasis, and worse prognosis. Loss of mismatch repair protein was correlated with loss of FHIT, and these FHIT-deficient cases showed a significant correlation with progression of carcinoma, as well as lymph node metastasis (70, 71). These findings suggest that mismatch repair protein may be important in maintaining the integrity of the common fragile locus within the *FHIT* gene, and FHIT plays a role in a small but significant fraction of colorectal cancers.

Renal cancers showed absent or reduced FHIT expression in 31.3% of the tumors, and there was a statistically significant correlation between loss of FHIT expression and increasing grade ($P = 0.006$) and more advanced stage ($P = 0.023$). An earlier study found that loss of FHIT expression was significantly less pronounced in poorly differentiated renal call carcinoma or advanced tumor stage (43). In this study, all the clear cell carcinomas studied showed a reduced FHIT expression, which was not observed in our cases in which normal FHIT staining was seen in 69% of the cases. We observed a moderate to strong intensity of FHIT staining in the kidney tumors, and in some cases, this matched the staining intensity of normal kidney tubules which were used as controls. In another study done on collecting duct carcinomas, FHIT expression was reduced in only 3 of the total 11 cases (73). Hadaczek et al. described a correlation between reduced FHIT expression and 3p allelic loss in renal carcinomas (74). Although in our study, FHIT inactivation does not seem to be a common event in renal cell carcinomas, the involvement of the *FHIT* gene in the tumorigenesis of this tumor cannot be ruled out.

Reduced or absent FHIT expression was seen in 45% of the liver cancers in this cohort from Saudi Arabia, where exposure to hepatic carcinogens is low. Loss of FHIT expression was more commonly observed in poorly differentiated hepatic tumors ($P = 0.027$) and associated with lymph node metastasis ($P = 0.0392$). Reduced or absent expression was seen in 75% of the grade 3 hepatocellular carcinomas as compared with only 22% in the grade 1 tumors. A positive, although not statistically significant, correlation was also noted between reduced FHIT expression and advanced stage. There was no difference in the survival analyses between the two groups ($P = 0.492$).

An earlier study on a cohort from the U.S., where the exposure to hepatic carcinogens is low, has showed a reduced expression of FHIT in 15% of the hepatic tumors (44). Marked reduction or absence of FHIT protein by immunohistochemistry staining has been reported in 65% of the 83 hepatocellular carcinomas examined from China, where loss was associated with increasing tumor size and stage (75). FHIT inactivation is probably a later event associated with hepatic carcinogenesis and might play only a minor role in hepatic carcinogenesis in Saudi Arabia.

FHIT expression was reduced or absent in 67.6% of the lung tumors, which is in concordance with earlier studies (28, 33, 76, 77). Tumors showing loss of FHIT expression were of a higher stage ($P = 0.031$), larger size ($P = 0.046$), and occurred more commonly in the older age group ($P = 0.006$). FHIT expression was also correlated with histology ($P < 0.006$). One hundred percent of the small cell carcinomas and 92% of the squamous cell carcinomas showed a reduced or absent FHIT expression as compared with 54% of adenocarcinomas or 25% of bronchioalveolar carcinomas. There was a positive correlation between loss of FHIT expression and older age,

which was, however, not statistically significant ($P = 0.061$). Similarly, there was no difference in the survival patterns ($P = 0.199$) between the two groups (normal versus reduced/absent FHIT expression). Extensive studies have been carried out due to the loss of *FHIT* gene following exposure to various carcinogens (76). Earlier studies have shown a correlation between loss of FHIT expression and a higher proliferation index and a lower apoptotic index; as well as an inverse correlation between loss of FHIT expression and patient survival (33). The same study stressed the important role of FHIT in carcinogenesis, especially squamous cell carcinomas, in association with smoking. In another study, there was no correlation between FHIT expression and a variety of clinical variables including survival and abnormal immunohistochemical expression of *p53, rb*, and *p16* (77). The same authors concluded that loss of FHIT expression is an extremely common and independent genetic abnormality that occurs independently of the metastatic state and other molecular abnormalities.

Loss of FHIT expression was seen in 65.8% of the non–Hodgkin's diffuse large B cell lymphoma. No correlation was seen between FHIT expression and age, sex, stage, and survival patterns. There is only one earlier study of FHIT expression by immunohistochemistry in diffuse large B cell lymphoma. In that study, polyclonal rabbit IgG anti-FHIT antibody was used at a dilution of 1:200 at an incubation time of only 10 minutes (45). This study was done on 31 patients and showed that decreased FHIT expression indicates a significantly bad prognosis in diffuse large B cell lymphomas.

We now briefly discuss the prevalence of FHIT expression in some tumors which were arrayed in the multitumor array block. Complete loss or reduced FHIT expression was seen in 24% of the total 34 gastric carcinomas in our study. Loss of FHIT is possibly an early event in gastric carcinoma and has been seen in 49% of the 55 gastric adenocarcinomas studied (41).

Loss of FHIT expression was seen in 54% of the bladder tumors. An earlier study had shown a loss of FHIT expression in 61% of the bladder cancers as well as a significant correlation between reduced FHIT expression and advanced stage of the disease. As for other neoplasms caused by environmental carcinogens, FHIT inactivation could play an important role as a late event in the development of bladder tumors (37). Loss of FHIT expression was seen in 63% of the skin tumors. Reduced FHIT expression was seen in 73% of the basal cell carcinomas and 70% of the squamous cell carcinomas. This suggests an early loss of *FHIT* gene in the progression of skin cancers. To the best of our knowledge, this is the first such study using immunohistochemistry to study FHIT expression in skin cancers. An earlier study had found a high frequency of *FHIT* gene abnormalities in Merkel cell carcinomas (78). Abnormal FHIT transcripts were seen in 57% of the cases.

Other authors studied some non–melanoma skin cancers (basal cell carcinoma, squamous cell carcinoma, and actinic keratosis) and concluded that the *FHIT* gene is not a very common target in skin cancers (79).

The meningiomas also showed reduced or absent expression in 15% of the cases. Normal FHIT expression was seen in 72% of the cases. Staining heterogeneity was seen in 12.5% of the cases in the fibroblastic, meningothelial, and transitional cell subtypes. To the best of our knowledge, no previous studies have been done to see *FHIT* gene abnormalities in meningiomas.

Loss of FHIT expression was seen in 80% of the soft tissue sarcomas. Surprisingly, all 17 cases of dermatofibrosarcoma protuberans, a benign tumor, showed complete loss of FHIT expression. In an earlier study by reverse transcription-PCR analysis of the *FHIT* gene, normal and abnormal FHIT transcripts were found in 11 (69%) of 16 osteosarcomas, and in 3 (27%) of 11 Ewing sarcomas (80).

Loss of FHIT expression was seen in 19.04% of the ovarian tumors. Earlier studies have shown the absence of FHIT protein by immunohistochemistry in 34% of the ovarian carcinomas (81), and concluded that FHIT probably plays a role in a small proportion of ovarian cancers (82). FHIT expression was reduced or absent in nasopharyngeal carcinomas (all 9 cases), in esophageal carcinoma (all 4 cases), and 64.7% of the head and neck cancers studied (17 cases). In an earlier study, low FHIT expression was seen in 53% of the head and neck squamous cell carcinomas and correlated with Ki-67 expression (83). In an earlier study, primary esophageal tumor (76%) showed loss of heterozygosity encompassing FHIT, and 70% were negative for FHIT protein (84). In this study, tumors from patients who were heavy users of tobacco and alcohol showed significantly higher frequencies of loss of FHIT expression. Noncancerous squamous epithelia were mostly positive for FHIT, but five samples from heavy tobacco/alcohol users were FHIT-negative. In addition, most carcinomas *in situ*, 50% of severe and moderate dysplasias, and 33% of mild dysplasia were FHIT-negative, suggesting that FHIT loss is an early event in esophageal squamous cell carcinoma development (84).

The data summarized indicates that the tumor suppressor gene, *FHIT*, is altered in almost all human tumors, particularly those caused by environmental carcinogens. In some of these tumors, such as lung cancer, in which FHIT loss occurs early in the carcinogenesis pathway, there is a correlation between FHIT loss and the clinical variables. However, in some tumors, loss of FHIT expression is associated with other factors in some tumors and the role of FHIT is still to be elucidated. Finally, there is a subgroup of tumors in which FHIT loss is seen only in a small fraction of the tumors and FHIT probably plays a very minor role in carcinogenesis (Fig. 2). We have also highlighted that ethnic differences exist in the loss of FHIT expression in tumors from Saudi Arabia, especially in breast carcinomas and liver carcinomas.

References

1. ar-Rushdi A, Nishikura K, Erikson J, et al. Differential expression of the translocated and the untranslocated c-myc oncogene in Burkitt lymphoma. Science 1983;222:390–3.
2. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science 1985;228:1440–3.
3. Arakawa H, Nakamura T, Zhadanov AB, et al. Identification and characterization of the ARP1 gene, a target for the human acute leukemia ALL1 gene. Proc Natl Acad Sci U S A 1998;95:4573–8.
4. Rimessi P, Gualandi F, Morelli C, et al. Transfer of human chromosome 3 to an ovarian carcinoma cell line identifies three regions on 3p involved in ovarian cancer. Oncogene 1994;9:3467–74.
5. Linehan WM, Lerman MI, Zbar B. Identification of the von Hippel-Lindau (VHL) gene. Its role in renal cancer. JAMA 1995;273:564–70.
6. Bronner CE, Baker SM, Morrison PT, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 1994;368:258–61.
7. Ohmura H, Tahara H, Suzuki M, et al. Restoration of the cellular senescence program and repression of telomerase by human chromosome 3. Jpn J Cancer Res 1995;86:899–904.
8. Ohta M, Inoue H, Cotticelli MG, et al. 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 1996;84:587–97.
9. Brenner C, Bieganowski P, Pace HC, Huebner K. The histidine triad superfamily of nucleotide-binding proteins. J Cell Physiol 1999;181:179–87.
10. Siprashvili Z, Sozzi G, Barnes LD, et al. Replacement of Fhit in cancer cells suppresses tumorigenicity. Proc Natl Acad Sci U S A 1997;94:13771–6.
11. Ji L, Fang B, Yen N, et al. Induction of apoptosis and inhibition of tumorigenicity and tumor growth by adenovirus vector-mediated fragile histidine triad (FHIT) gene overexpression. Cancer Res 1999;59:3333–9.
12. Sard L, Accornero P, Tornielli S, et al. The tumor-suppressor gene FHIT is involved in the regulation of apoptosis and in cell cycle control. Proc Natl Acad Sci U S A 1999;96:8489–92.
13. Fong KM, Biesterveld EJ, Virmani A, et al. FHIT and FRA3B 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 1997;57:2256–67.
14. Fullwood P, Marchini S, Rader JS, et al. Detailed genetic and physical mapping of tumor suppressor loci on chromosome 3p in ovarian cancer. Cancer Res 1999;59:4662–7.
15. Guo Z, Wu F, Asplund A, et al. Analysis of intratumoral heterogeneity of chromosome 3p deletions and genetic evidence of polyclonal origin of cervical squamous carcinoma. Mod Pathol 2001;14:54–61.
16. Kastury K, Baffa R, Druck T, et al. Potential gastrointestinal tumor suppressor locus at the 3p14.2 FRA3B site identified by homozygous deletions in tumor cell lines. Cancer Res 1996;56:978–83.
17. Maitra A, Wistuba II, Washington C, et al. High-resolution chromosome 3p allelotyping of breast carcinomas and precursor lesions demonstrates frequent loss of heterozygosity and a discontinuous pattern of allele loss. Am J Pathol 2001;159:119–30.
18. Negrini M, Monaco C, Vorechovsky I, et al. The FHIT gene at 3p14.2 is abnormal in breast carcinomas. Cancer Res 1996;56:3173–9.
19. Sozzi G, Veronese ML, Negrini M, et al. The FHIT gene 3p14.2 is abnormal in lung cancer. Cell 1996;85:17–26.
20. Sozzi G, Sard L, De Gregorio L, et al. Association between cigarette smoking and FHIT gene alterations in lung cancer. Cancer Res 1997;57:2121–3.
21. Sozzi G, Tornielli S, Tagliabue E, et al. Absence of Fhit protein in primary lung tumors and cell lines with FHIT gene abnormalities. Cancer Res 1997; 57:5207–12.
22. Velickovic M, Delahunt B, Grebe SK. Loss of heterozygosity at 3p14.2 in clear cell renal cell carcinoma is an early event and is highly localized to the FHIT gene locus. Cancer Res 1999;59:1323–6.
23. Virgilio L, Shuster M, Gollin SM, et al. FHIT gene alterations in head and neck squamous cell carcinomas. Proc Natl Acad Sci U S A 1996;93:9770–5.
24. Wu R, Connolly DC, Dunn RL, Cho KR. Restored expression of fragile histidine triad protein and tumorigenicity of cervical carcinoma cells. J Natl Cancer Inst 2000;92:338–44.
25. Burke L, Khan MA, Freedman AN, et al. Allelic deletion analysis of the FHIT gene predicts poor survival in non-small cell lung cancer. Cancer Res 1998; 58:2533–6.
26. Garinis GA, Gorgoulis VG, Mariatos G, et al. Association of allelic loss at the FHIT locus and p53 alterations with tumour kinetics and chromosomal instability in non-small cell lung carcinomas (NSCLCs). J Pathol 2001;193: 55–65.
27. Marchetti A, Pellegrini S, Bertacca G, et al. FHIT and p53 gene abnormalities in bronchioloalveolar carcinomas. Correlations with clinicopathological data and K-ras mutations. J Pathol 1998;184:240–6.
28. Pavelic K, Krizanac S, Cacev T, et al. Aberration of FHIT gene is associated with increased tumor proliferation and decreased apoptosis-clinical evidence in lung and head and neck carcinomas. Mol Med 2001;7: 442–53.
29. Sozzi G, Musso K, Ratcliffe C, et al. Detection of microsatellite alterations in plasma DNA of non-small cell lung cancer patients: a prospect for early diagnosis. Clin Cancer Res 1999;5:2689–92.
30. Tokuchi Y, Kobayashi Y, Hayashi S, et al. Abnormal FHIT transcripts found in both lung cancer and normal lung tissue. Genes Chromosomes Cancer 1999;24:105–11.
31. Tseng JE, Kemp BL, Khuri FR, et al. Loss of Fhit is frequent in stage I non-small cell lung cancer and in the lungs of chronic smokers. Cancer Res 1999; 59:4798–803.
32. Wistuba II, Behrens C, Virmani AK, et al. High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. Cancer Res 2000;60: 1949–60.
33. Toledo G, Sola JJ, Lozano MD, Soria E, Pardo J. Loss of FHIT protein expression is related to high proliferation, low apoptosis and worse prognosis in non-small-cell lung cancer. Mod Pathol 2004;17:440–8.
34. Ginestier C, Bardou VJ, Popovici C, et al. Loss of FHIT protein expression is a marker of adverse evolution in good prognosis localized breast cancer. Int J Cancer 2003;107:854–62.
35. Gatalica Z, Lele SM, Rampy BA, Norris BA. The expression of Fhit protein is related inversely to disease progression in patients with breast carcinoma. Cancer 2000;88:1378–83.
36. Campiglio M, Pekarsky Y, Menard S, et al. FHIT loss of function in human primary breast cancer correlates with advanced stage of the disease. Cancer Res 1999;59:3866–9.
37. Baffa R, Gomella LG, Vecchione A, et al. Loss of FHIT expression in transitional cell carcinoma of the urinary bladder. Am J Pathol 2000;156: 419–24.
38. Kisielewski AE, Xiao GH, Liu SC, et al. Analysis of the FHIT gene and its product in squamous cell carcinomas of the head and neck. Oncogene 1998; 17:83–91.
39. Michael D, Beer DG, Wilke CW, Miller DE, Glover TW. Frequent deletions of FHIT and FRA3B in Barrett's metaplasia and esophageal adenocarcinomas. Oncogene 1997;15:1653–9.
40. Tanaka H, Shimada Y, Harada H, et al. Methylation of the 5′ CpG island of the FHIT gene is closely associated with transcriptional inactivation in esophageal squamous cell carcinomas. Cancer Res 1998;58:3429–34.
41. Capuzzi D, Santoro E, Hauck WW, et al. Fhit expression in gastric adenocarcinoma: correlation with disease stage and survival. Cancer 2000; 88:24–34.
42. Hao XP, Willis JE, Pretlow TG, et al. Loss of fragile histidine triad expression in colorectal carcinomas and premalignant lesions. Cancer Res 2000;60:18–21.
43. Ramp U, Caliskan E, Ebert T, et al. FHIT expression in clear cell renal carcinomas: versatility of protein levels and correlation with survival. J Pathol 2002;196:430–6.
44. Kannangai R, Sahin F, Adegbola O, et al. FHIT mRNA and protein expression in hepatocellular carcinoma. Mod Pathol 2004;17:653–9.
45. Chen PM, Yang MH, Hsiao LT, et al. Decreased FHIT protein expression correlates with a worse prognosis in patients with diffuse large B-cell lymphoma. Oncol Rep 2004;11:349–56.
46. Simon R, Sauter G. Tissue microarrays for miniaturized high-throughput molecular profiling of tumors. Exp Hematol 2002;30:1365–72.
47. Greenspan DL, Connolly DC, Wu R, et al. Loss of FHIT expression in cervical carcinoma cell lines and primary tumors. Cancer Res 1997;57: 4692–8.
48. Ozaki K, Enomoto T, Yoshino K, et al. fhit Alterations in endometrial carcinoma and hyperplasia. Int J Cancer 2000;85:306–12.
49. Helland A, Kraggerud SM, Kristensen GB, et al. Primary cervical carcinomas show 2 common regions of deletion at 3P, 1 within the FHIT gene: evaluation of allelic imbalance at FHIT, RB1 and TP53 in relation to survival. Int J Cancer 2000;88:217–22.
50. Druck T, Berk L, Huebner K. FHITness and cancer. Oncol Res 1998;10:341–5.
51. Knudson AG, Jr. Hereditary cancer, oncogenes, and antioncogenes. Cancer Res 1985;45:1437–43.
52. Vogelstein B, Kinzler KW. The multistep nature of cancer. Trends Genet 1993;9:138–41.
53. Huebner K, Garrison PN, Barnes LD, Croce CM. The role of the FHIT/FRA3B locus in cancer. Annu Rev Genet 1998;32:7–31.
54. Roz L, Gramegna M, Ishii H, Croce CM, Sozzi G. Restoration of fragile histidine triad (FHIT) expression induces apoptosis and suppresses tumorigenicity in lung and cervical cancer cell lines. Proc Natl Acad Sci U S A 2002;99:3615–20.
55. Barnes LD, Garrison PN, Siprashvili Z, et al. Fhit, a putative tumor suppressor in humans, is a dinucleoside 5′,5‴-P1,P3-triphosphate hydrolase. Biochemistry 1996;35:11529–35.
56. Trapasso F, Krakowiak A, Cesari R, et al. Designed FHIT alleles establish that Fhit-induced apoptosis in cancer cells is limited by substrate binding. Proc Natl Acad Sci U S A 2003;100:1592–7.
57. Panagopoulos I, Thelin S, Mertens F, Mitelman F, Aman P. Variable FHIT transcripts in non-neoplastic tissues. Genes Chromosomes Cancer 1997;19: 215–9.
58. Pekarsky Y, Zanesi N, Palamarchuk A, Huebner K, Croce CM. FHIT: from gene discovery to cancer treatment and prevention. Lancet Oncol 2002;3: 748–54.
59. Huebner K, Croce CM. Cancer and the FRA3B/FHIT fragile locus: it's a HIT. Br J Cancer 2003;88:1501–6.
60. Dumon KR, Ishii H, Fong LY, et al. FHIT gene therapy prevents tumor development in Fhit-deficient mice. Proc Natl Acad Sci U S A 2001;98: 3346–51.
61. Ishii H, Zanesi N, Vecchione A, et al. Regression of upper gastric cancer in mice by FHIT gene delivery. FASEB J 2003;17:1768–70.
62. Ishii H, Ozawa K, Furukawa Y. Alteration of the fragile histidine triad gene early in carcinogenesis: an update. J Exp Ther Oncol 2003;3:291–6.
63. Butler D, Collins C, Mabruk M, et al. Deletion of the FHIT gene in neoplastic and invasive cervical lesions is related to high-risk HPV infection but is independent of histopathological features. J Pathol 2000;192:502–10.
64. Baykal C, Ayhan A, Al A, Yuce K, Ayhan A. No relationship is indicated between FHIT expression and clinicopathologic prognostic parameters in early stage cervical carcinoma. Int J Gynecol Cancer 2003;13:192–6.
65. Kantarjian HM, Talpaz M, O'Brien S, et al. Significance of FHIT expression in chronic myelogenous leukemia. Clin Cancer Res 1999;5:4059–64.
66. Went PT, Dirnhofer S, Bundi M, et al. Prevalence of KIT expression in human tumors. J Clin Oncol 2004;22:4514–22.
67. Schraml P, Bucher C, Bissig H, et al. Cyclin E overexpression and amplification in human tumours. J Pathol 2003;200:375–82.
68. Yang Q, Yoshimura G, Suzuma T, et al. Clinicopathological significance of fragile histidine triad transcription protein expression in breast carcinoma. Clin Cancer Res 2001;7:3869–73.
69. Al-Kuraya K, Schraml P, Sheikh S, et al. Predominance of high-grade pathway in breast cancer development of Middle East women. Mod Pathol 2005;18:891–7.
70. Mori M, Mimori K, Masuda T, et al. Absence of Msh2 protein expression is associated with alteration in the FHIT locus and Fhit protein expression in colorectal carcinoma. Cancer Res 2001;61:7379–82.
71. Andachi H, Yashima K, Koda M, et al. Reduced Fhit expression is associated with mismatch repair deficiency in human advanced colorectal carcinoma. Br J Cancer 2002;87:441–5.
72. Mady HH, Melhem MF. FHIT protein expression and its relation to apoptosis, tumor histologic grade and prognosis in colorectal adenocarcinoma: an immunohistochemical and image analysis study. Clin Exp Metastasis 2002;19:351–8.
73. Vecchione A, Galetti TP, Gardiman M, et al. Collecting duct carcinoma of the kidney: an immunohistochemical study of 11 cases. BMC Urol 2004;4:11.
74. Hadaczek P, Kovatich A, Gronwald J, et al. Loss or reduction of Fhit expression in renal neoplasias: correlation with histogenic class. Hum Pathol 1999;30:1276–83.

75. Zhao P, Song X, Nin YY, Lu YL, Li XH. Loss of fragile histidine triad protein in human hepatocellular carcinoma. World J Gastroenterol 2003;9:1216–9.
76. Pylkkanen L, Wolff H, Stjernvall T, et al. Reduced Fhit protein expression and loss of heterozygosity at FHIT gene in tumours from smoking and asbestos-exposed lung cancer patients. Int J Oncol 2002;20:285–90.
77. Geradts J, Fong KM, Zimmerman PV, Minna JD. Loss of Fhit expression in non-small-cell lung cancer: correlation with molecular genetic abnormalities and clinicopathological features. Br J Cancer 2000;82:1191–7.
78. Zanesi N, Croce CM. Fragile histidine triad gene and skin cancer. Eur J Dermatol 2001;11:401–4.
79. Sikkink SK, Rehman I, Rees JL. Deletion mapping of chromosome 3p and 13q and preliminary analysis of the FHIT gene in human nonmelanoma skin cancer. J Invest Dermatol 1997;109:801–5.
80. Hinohara S, Satake N, Sekine K, Kaneko Y. Abnormalities of the FHIT transcripts in osteosarcoma and Ewing sarcoma. Jpn J Cancer Res 1998;89:887–94.
81. Quddus MR, Sung CJ, Cook SW, et al. Loss of Fhit protein in carcinoma of primary and secondary mullerian systems. Histopathology 2004;44:87–8.
82. Buttitta F, Marchetti A, Radi O, et al. Evaluation of FHIT gene alterations in ovarian cancer. Br J Cancer 1998;77:1048–51.
83. Mineta H, Miura K, Takebayashi S, et al. Low expression of fragile histidine triad gene correlates with high proliferation in head and neck squamous cell carcinoma. Oral Oncol 2003;39:56–63.
84. Mori M, Mimori K, Shiraishi T, et al. Altered expression of Fhit in carcinoma and precarcinomatous lesions of the esophagus. Cancer Res 2000;60:1177–82.