Modern Pathology (2006) 19, 1124–1129
© 2006 USCAP, Inc All rights reserved 0893-3952/06 $30.00
www.modernpathology.org

High throughput tissue microarray analysis of *FHIT* expression in diffuse large cell B-cell lymphoma from Saudi Arabia

Khawla Al Kuraya[1], Abdul Khalid Siraj[1], Prashant Bavi[1], Naif Al-Jomah[1], Hassan El-Solh[1], Adnan Ezzat[1], Fouad Al-Dayel[1], Asim Belgaumi[1], Amani Al-Kofide[1], Rajeh Sabbah[1], Salwa Sheikh[2], Samir Amr[2], Ronald Simon[3] and Guido Sauter[3]

[1]*Cancer Genomics, Research Centre, King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia;* [2]*Pathology Services Division, Saudi Aramco, Dhahran, Saudi Arabia and* [3]*Department of Pathology, University Medical Center Hamburg Eppendorf, Hamburg, Germany*

Recent studies have suggested a potential prognostic role of alterations of the *fragile histidine triad* (*FHIT*) gene in diffuse large B-cell lymphoma. To evaluate possible mechanisms of *FHIT* inactivation and to further clarify its potential prognostic relevance, we analyzed a set of 114 diffuse large B-cell lymphoma with clinical follow-up information. Tissue microarrays were analyzed by immunohistochemistry for protein expression, and corresponding DNA samples were analyzed for *FHIT* promotor hypermethlyation. Reduced or absent *FHIT* expression was found in 75 of 114 diffuse large B-cell lymphoma (66%), but was unrelated to clinical tumor stage or patient prognosis. *FHIT* promotor hypermethylation was observed in 29 of 93 (23%) interpretable diffuse large B-cell lymphoma. Hypermethylation was not significantly correlated to protein expression loss, which could be explained by competing mechanisms for *FHIT* inactivation in a substantial fraction of non *FHIT* hypermethylated diffuse large B-cell lymphoma. Hypermethylation was significantly associated with poor prognosis of diffuse large B-cell lymphoma patients and predominantly seen in nongerminal center diffuse large B-cell lymphoma (27%), but less frequent (13%) in germinal center diffuse large B-cell lymphoma. In summary, these data suggest that promotor hypermethylation is responsible for reduced *FHIT* expression in a substantial subset of diffuse large B-cell lymphoma, which is primarily composed of nongerminal center subtype with poor patient prognosis.
Modern Pathology (2006) **19,** 1124–1129. doi:10.1038/modpathol.3800631; published online 19 May 2006

Keywords: lymphoma genetics; FHIT; methylation

Diffuse large B-cell lymphoma is the most common subtype of non-Hodgkin's lymphoma and accounts for 30–40% of new diagnoses.[1] Prognosis of diffuse large B-cell lymphoma patients is poor. Despite multiagent chemotherapy, durable remissions are achieved in only 40–50% of patients. Current attempts to determine prognosis in diffuse large B-cell lymphoma rely on clinical parameters, but are still not reliable enough to predict the course of the disease in individual patients.[2] It is hoped that a better understanding of the molecular basis of the disease will eventually lead to better prognostic markers. Indeed, several new proteins or groups of genes playing a role in prognosis or that may potentially serve as therapeutic targets have recently been discovered.[3–6]

The *fragile histidine triad* (*FHIT*) gene located on chromosome 3p14.2 at fragile site, FRA3B, belongs to these genes that have recently been linked to diffuse large B-cell lymphoma.[7,8] *FHIT* is known to be inactivated in various human malignancies.[9–20] *FHIT* inactivation by point mutation is a rare event,[21–23] but significant loss or reduction of expression can be caused by other mechanisms, including loss of heterozygosity (LOH) and/or promoter hypermethylation.[24] For example, *FHIT* hypermethylation with consequent transcriptional inactivation has been shown in breast, lung, esophageal, cervical, prostate and bladder cancer.[25–29] For breast cancer, it was demonstrated that hypermethylation of one allele can occur in conjunction with LOH, and that these two events can constitute the 'two hits' required for the complete gene silencing.[30]

Correspondence: Dr R Simon, PhD, Department of Pathology, University Medical Center Hamburg Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany.
E-mail: r.simon@uke.uni-hamburg.de
Received 19 September 2005; revised and accepted 13 April 2006; published online 19 May 2006

A recent immunohistochemistry study on 31 diffuse large B-cell lymphoma patients had suggested that decreased or absent *FHIT* protein expression may herald poor prognosis in diffuse large B-cell lymphoma.[7] More recently, it was shown that microdeletions within the *FHIT* gene result in the selective loss of certain exons, which can cause aberrant RNA expression in diffuse large B-cell lymphoma. Other mechanisms of reduced *FHIT* expression have not been analyzed in diffuse large B-cell lymphoma. The aims of this study were therefore two-fold. First, we aimed at a confirmation of the prognostic relevance of reduced *FHIT* expression in a series of >100 diffuse large B-cell lymphoma. Second, we investigated the role of promotor methylation status for *FHIT* inactivation. Overall, our data confirm a major role of *FHIT* alteration in the pathogenesis of diffuse large B-cell lymphoma.

Materials and methods

Tissue Samples

Formalin-fixed, paraffin-embedded samples from 190 newly presenting and previously untreated patients with diffuse large B-cell lymphoma were investigated. Diagnosis was confirmed by pathologic review using the diagnostic criteria defined in the revised European-American Classification Lymphoid Neoplasms/WHO Classification.[31] Clinical follow-up information was available from all patients. Study approval was obtained from the Research Advisory Council (RAC #2030 019) at King Faisal Specialist Hospital and Research Centre. Tissue microarry construction was as described.[32] Briefly, tissue cylinders with a diameter of 0.6 mm were then punched from representative tumor regions of each 'donor' tissue block and brought into a recipient paraffin block using a home made semiautomated precision instrument.

Methylation-Specific Polymerase Chain Recation Analysis

For methylation-specific polymerase chain reaction analysis, genomic DNA was either extracted with a Puregene kit (Gentra, Minneapolis, MN, USA) or was available from previous studies.[29,33] One microgram of genomic DNA was denatured in 0.4 M NaOH and modified with 3 M sodium bisulfite and 10 mM hydroquinone at 55°C for 16 h. After purification with a GeneCleanIII kit (Bio 101, Vista, CA, USA), the DNA was desulfonated in 0.4 M NaOH, precipitated in ethanol, and resuspended in dH_2O. Then 200 ng was used as a template in methylation-specific polymerase chain reactions with 1.5 mM $MgCl_2$ and 20 pmol of primers specific for methylated (M) and unmethylated (U) forms.[25] The methylated *FHIT* reaction consisted of 32 cycles of touchdown PCR at annealing ranging from 71 to 63°C with primers TGGGGCGCGGGTTTGGGTTTTTACGC and CGTAAACGACGCCGACCCCACTA. The unmethylated *FHIT* reaction was done at 64°C for 33 cycles with primers TTGGGGTGTGGGTTTGGGTTTTTATG and CATAAACAACACCAACCCCACTA, corresponding to nucleotides 189–301 (GenBank Accession Number U76263). Each reaction was tested with untreated DNA to ensure lack of amplification, and three controls were included to ensure specificity: (1) normal human DNA previously treated with the CpG methylase SSS1 in the presence of *S*-adenosylmethionine (*in vitro* methylated DNA); (2) DNA from peripheral lymphocytes from a healthy individual (normal control); and (3) no template (blank). PCR products were analyzed after electrophoresis on 4% agarose gels containing ethidium bromide.

Immunohistochemical Staining for *FHIT* Protein

Paraffin-embedded 5 μm sections from the tissue microarry block were stained for *FHIT* protein, according to the method described by Yang *et al*.[34] Briefly, paraffin embedded sections on polylysine coated slides were dewaxed with xylene and rehydrated through a graded alcohol series. Endogenous peroxidase activity was blocked in 3% hydrogen peroxidase in methanol for 10 min. Antigen retrieval was performed by placing the sides in a Citrate buffer (pH 6.0) and microwaving them for 5 min at 750 W and for 15 min at 250 W. The sections were incubated for 90 min in 1:900 dilutions of polyclonal rabbit antibodies reacting against *FHIT* protein (ZR44 Zymed, USA). Bound antibody was detected with biotinylated link antibody (Dako, Glostrup, Danmark) and horse radish peroxidase labeled streptavidin (Dako). The reaction was developed in 3,3′-diamino benzidine with H_2O_2 as substrate (Dako). The sections were then counterstained with Gills hematoxylin. The primary antibody was omitted in negative control sections.

Expression was scored on a four tiered scale for both intensity (grade 0, no staining; grade 1, 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. Score 0 was defined as absent or lost expression, scores 1–3 were defined as markedly reduced *FHIT* expression and scored 4–9 were considered as normal expression.[35–37]

Statistical Analysis

Statistical analysis was performed using SAS's (SAS Institute Inc.) JMP 5.1 software (Cary, NC, USA), and all *P*-values reported are two-tailed. Univariate analysis of categorical variables was conducted using contingency analysis and χ^2 tests. Surviving

curves were plotted according to the Kaplan–Meier method. Survival differences between groups were analyzed by log-rank test.

Results

FHIT Immunohistochemistry

Immunohistochemical staining for *FHIT* protein expression was successful in 114 of 190 diffuse large B-cell lymphoma. The absence of tissue or lack of clearly discernible tumor cells were the cause of noninformative results in 76 additional cases. Out of 114 informative cases, 39 (34%) showed strong (Figure 1c), 57 (50%) weak (Figure 1b), and 18 (16%) absent *FHIT* staining (Figure 1a), according to our definition. No survival difference was seen between diffuse large B-cell lymphoma with different *FHIT* expression level.

FHIT Methylation

FHIT promoter hypermethylation analysis was successful in 93 of 114 diffuse large B-cell lymphoma with available immunohistochemistry data (82%). Unsuccessful analyses were due to insufficient DNA quality in 21 cases. *FHIT* hypermethylation was found in 29 (23%) of 93 interpretable samples (Figure 2). *FHIT* methylation was unrelated to lymphoma stage (Table 1), but was significantly associated with short patient survival $P = 0.023$ (Figure 3). A comparison of methylation and immunohistochemistry data revealed methylation in 15 of 59 (25%) cases with absent or reduced *FHIT* expression by immunohistochemistry and in six of 34 (17%) tumors with normal *FHIT* expression. This association was statistically not significant.

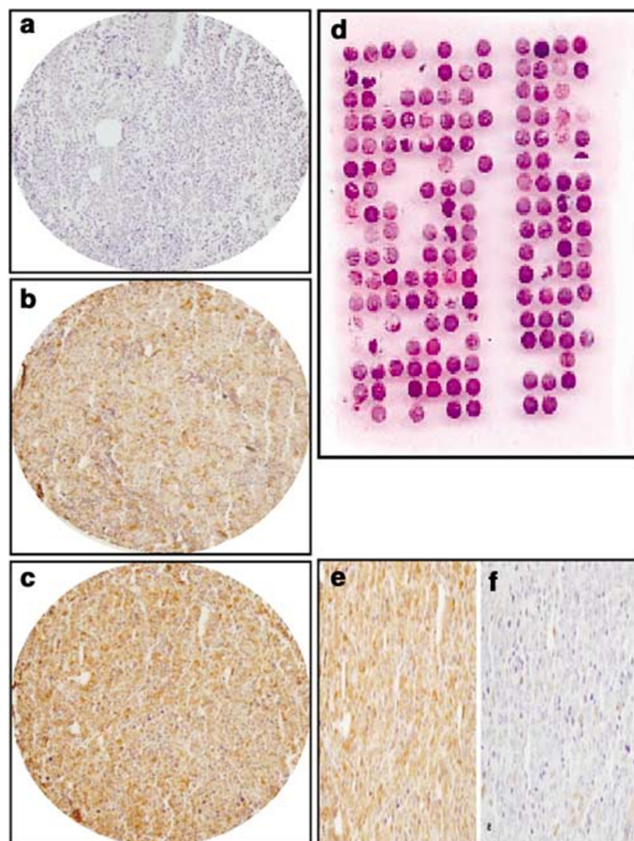

Figure 1 Diffuse large B-cell lymphoma tissue microarry. (**a**) absent *fhit* expression, ×10; (**b**) reduced *fhit* expression, ×10; (**c**) normal *fhit* expression, ×10; (**d**) H&E stained tissue micro array of diffuse large B-cell lymphoma; (**e**) normal *fhit* expression, ×40; and (**f**) absent *fhit* expression, ×40.

Relationship to Diffuse Large B-Cell Lymphoma Subtype

CD10 and bcl6 immunohistochemistry to define germinal center (CD10/bcl6 positive) and nongerminal center (CD10/bcl6 negative) diffuse large B-cell lymphoma subtypes had previously been performed in our tumors.[38] This analysis had unequivocally identified eight germinal center (CD10/bcl6 positive) and 45 nongerminal center (CD10/bcl6 negative) in our 114 interpretable diffuse large B-cell lymphoma. Remarkably, our comparison of *FHIT* results and diffuse large B-cell lymphoma phenotype revealed discrepant results for methylation and immunohistochemistry data. Methylation results showed a tendency towards more *FHIT* methylation in nongerminal center phenotype (12 of 45; 27%) than in germinal center phenotype (1 of 8; 13%). At the same time, the immunohistochemistry data suggested expression loss to be more frequent in germinal center (reduced in nine of 10 cases, 90%) than in nongerminal center (reduced in 35 of 57, 61%) phenotype ($P = 0.05$).

Discussion

Our data suggest that promotor hypermethylation contributes to *FHIT* downregulation in diffuse large B-cell lymphoma. This is of potential clinical importance as new treatment regimens targeting and reversing hypermethylation of *FHIT* are now in clinical trials. *FHIT* belongs to the most commonly altered genes in all human cancers, and is believed to be inactivated in 20–100% (depending on the

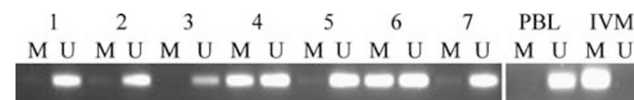

Figure 2 Methylation analysis. Methylation-specific PCR analyses of seven representative NHL samples (labeled 1–7 on the top) including normal PBL as positive control for unmethylated reacation and *in vitro* methylase treated (IVM) DNA as positive control for methylated reaction. Both methylated (M) and unmethylated (U) reactions were amplified for each bisulfite-treated DNA and run in a 4% agarose gel.

Table 1 FHIT expression by IHC, methylation status of FHIT protein and characteristics of patient with diffuse large B-cell lymphoma

	FHIT methylation analysis			*FHIT protein expression level (IHC)*		
	n[a]	*Methylated (%)*	P-*value*	n[a]	*Reduced/lost (%)*[b]	P-*value*
Age (years)						
≤25	10	30	0.3015	18	83	0.0025
25–50	41	15		47	62	
50–75	38	26		44	71	
>75	4	50		5	0	
Stage						
1	12	17	0.1577	12	67	0.5985
2	28	11		28	72	
3	2	0		2	67	
4	3	67		3	40	
Subtype						
Germinal center	8	13	0.3619	10	90	0.0558
Nongerminal center	45	27		57	61	

[a]Analyzable cases only.
[b]Reduced = combined IHC score 1–3; absent = IHC score 0.

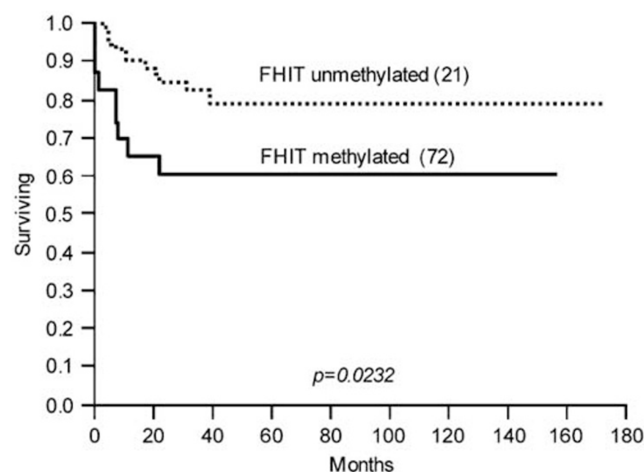

Figure 3 Prognostic significance of *FHIT* promoter methylation in diffuse large B-cell lymphoma.

tumor type) of neoplasias (reviewed in Pekarsky *et al*[39]). A potential efficacy of such drugs is supported by findings from clinical trials in various solid tumors,[40] for example, non-small-cell lung cancers[41] and squamous cell carcinomas of the cervix.[42] If such drugs should proof to be efficient in humans, about one-third of diffuse large B-cell lymphoma patients could potentially benefit from such treatments.

The exact molecular mechanism or functional pathway mediating *FHIT*'s tumor suppressor action is still not fully understood. It is known that *FHIT* hydrolases diadenosine nucleotides into ADP and AMP, but since this enzymatic activity does not seem to be required for its tumor-suppressor function there must be other relevant features of the protein,[12] that is regulation of apoptosis. It has been demonstrated that restoration of *FHIT* expression in lung and cervical cancer cell lines resulted in efficient induction of apoptosis and suppression of tumorigenicity, and that the apoptotic mechanism seems to be FADD (Fas associated via death domain) dependent, caspase-8 mediated and independent from regulation through Bcl-2 or Bcl-xl.[43,44] Most recent, it has bee shown that *FHIT* modulates the Pi3k/AKT pathway by downregulation of the anti-apoptotic survivin, an inhibitor of apoptosis protein (IAP) family member.[45]

Our data indicate that inactivation of *FHIT* might be due to different reasons. A comparison of expression data as observed by immunohistochemistry and promotor hypermethylation did not show a significant association. The much higher frequency of expression loss (66%) as compared to hypermethylation (23%) raises the possibility that other mechanisms than hypermethylation may reduce *FHIT* expression in most diffuse large B-cell lymphoma. Small deletions that selectively eliminate individual *FHIT* exons have recently been found in about 30% of diffuse large B-cell lymphoma.[8] As the epitope where the antibody binds is not known, it cannot be excluded that microdeletions may constitute another main reason for reduced expression as detected by our antibody. Discrepancies between immunohistochemistry and methylation analysis also included a small number of cases ($n = 6$) with normal *FHIT* expression but hypermethylation. Tumor heterogeneity for methylation is a possible explanation for these cases. In addition, it is presumed that methylation usually occurs monoallelic, and complete loss of expression is a consequence of a combination of methylation and allelic loss.[25] Finally, technical immunohistochemistry problems, including variable immunoreactivity because of different fixation conditions, might have contributed to the discrepant findings. For example,

we used the same antibodies as described by Chen et al,[7] but found a slightly higher fraction (65%) of cases with reduced expression as compared to the 58% in Chen's description.

Inherent limitations of the tissue microarry approach could also have contributed to the relatively high number of FHIT negative cases in this study. Focal reduction of immunoreactivity or biologic heterogeneity can lead to false negative immunostainings on tissue microarrys. It has been shown, however, that some of the disadvantages caused by the small size of samples analyzed on a tissue microarry will be compensated by the maximal standardization of tissue microarry analysis and interpretation.[46] For example, in one previous study, the prognostic significance of p53 positivity in breast cancer was identified on several different tissue microarrys manufactured from a series of >500 cancers but not on corresponding large sections.[47] This study shows that at least in some instances, tissue microarrys composed of one 0.6 mm sample per tumor can be superior over traditional large sections for identification of prognostic biomarkers.

A true prognostic role of *FHIT* inactivation in diffuse large B-cell lymphoma could be supported by the significant association observed between *FHIT* hypermethylation and short survival. PCR based hypermetylation analysis clearly is a more robust and reproducible method than immunohistochemistry, which is prone to numerous technical shortcomings.[48,49] However, our data also raise the possibility that certain *FHIT* inactivation mechanisms could be linked to different diffuse large B-cell lymphoma subtypes. With the exception of one case, *FHIT* hypermethylation was only seen in the nongerminal center diffuse large B-cell lymphoma subtype. Thus, the poor prognosis observed for *FHIT* methylated diffuse large B-cell lymphoma could be explained by the generally poor prognosis of nongerminal center diffuse large B-cell lymphoma previously reported in both Western[2] and Saudi patients.[38] Remarkably, such a tendency to an association with nongerminal center subtype was not found for reduced *FHIT* protein expression. In contrary, there was even a clear tendency towards a lower frequency of reduced *FHIT* expression in nongerminal center (60%) as compared to germinal center (88%) diffuse large B-cell lymphoma subtype. Although the respective P-values did not reach significance ($P = 0.1$) and the reliability of immunohistochemistry analysis is to some extent limited, this result raises the possibility that hypermethylation is primarily inactivating *FHIT* in nongerminal center diffuse large B-cell lymphoma while other mechanisms apply for *FHIT* inactivation in the germinal center diffuse large B-cell lymphoma subtype.

In summary, our data show that hypermethylation is a relevant mechanism for *FHIT* inactivation in diffuse large B-cell lymphoma and suggest a link of hypermethylation to nongerminal center subtype and poor prognosis. If methylated *FHIT* should indeed constitute a suitable therapeutic target, diffuse large B-cell lymphoma patients could substantially benefit from such new drugs. Overall, the accumulating data on DNA level *FHIT* alterations provide strong evidence for an important role of *FHIT* in development or progression in diffuse large B-cell lymphoma.

References

1 A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood 1997;89:3909–3918.
2 Shipp MA, Harrington DP, Anderson JR. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 1993;329:987–994.
3 Alizadeh AA, Eisen MB, Davis RE, *et al.* Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503–511.
4 Shipp MA, Ross KN, Tamayo P, *et al.* Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. Nat Med 2002;8:68–74.
5 Colomo L, Lopez-Guillermo A, Perales M, *et al.* Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. Blood 2003;101:78–84.
6 Chang CC, McClintock S, Cleveland RP, *et al.* Immunohistochemical expression patterns of germinal center and activation B-cell markers correlate with prognosis in diffuse large B-cell lymphoma. Am J Surg Pathol 2004;28:464–470.
7 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;11:349–356.
8 Kameoka Y, Tagawa H, Tsuzuki S, *et al.* Contig array CGH at 3p14.2 points to the *FRA3B/FHIT* common fragile region as the target gene in diffuse large B-cell lymphoma. Oncogene 2004;23:9148–9154.
9 Hendricks DT, Taylor R, Reed M, *et al.* *FHIT* gene expression in human ovarian, endometrial, and cervical cancer cell lines. Cancer Res 1997;57: 2112–2115.
10 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;56:978–983.
11 Michael D, Beer DG, Wilke CW, *et al.* Frequent deletions of *FHIT* and FRA3B in Barrett's metaplasia and esophageal adenocarcinomas. Oncogene 1997;15: 1653–1659.
12 Siprashvili Z, Sozzi G, Barnes LD, *et al.* Replacement of *Fhit* in cancer cells suppresses tumorigenicity. Proc Natl Acad Sci USA 1997;94:13771–13776.
13 Sozzi G, Veronese ML, Negrini M, *et al.* The *FHIT* gene 3p14.2 is abnormal in lung cancer. Cell 1996;85:17–26.
14 Virgilio L, Shuster M, Gollin SM, *et al.* *FHIT* gene alterations in head and neck squamous cell carcinomas. Proc Natl Acad Sci USA 1996;93:9770–9775.

15 Negrini M, Monaco C, Vorechovsky I, *et al.* The *FHIT* gene at 3p14.2 is abnormal in breast carcinomas. Cancer Res 1996;56:3173–3179.
16 Baffa R, Gomella LG, Vecchione A, *et al.* Loss of *FHIT* expression in transitional cell carcinoma of the urinary bladder. Am J Pathol 2000;156:419–424.
17 Simon B, Bartsch D, Barth P, *et al.* Frequent abnormalities of the putative tumor suppressor gene *FHIT* at 3p14.2 in pancreatic carcinoma cell lines. Cancer Res 1998;58:1583–1587.
18 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;57:2256–2267.
19 Hayashi S, Tanimoto K, Hajiro-Nakanishi K, *et al.* Abnormal *FHIT* transcripts in human breast carcinomas: a clinicopathological and epidemiological analysis of 61 Japanese cases. Cancer Res 1997;57: 1981–1985.
20 Sozzi G, Alder H, Tornielli S, *et al.* Aberrant *FHIT* transcripts in Merkel cell carcinoma. Cancer Res 1996;56:2472–2474.
21 Druck T, Hadaczek P, Fu TB, *et al.* Structure and expression of the human *FHIT* gene in normal and tumor cells. Cancer Res 1997;57:504–512.
22 Gemma A, Hagiwara K, Ke Y, *et al. FHIT* mutations in human primary gastric cancer. Cancer Res 1997;57:1435–1437.
23 Huebner K, Croce CM. FRA3B and other common fragile sites: the weakest links. Nat Rev Cancer 2001;1:214–221.
24 Esteller M. CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. Oncogene 2002;21:5427–5440.
25 Zochbauer-Muller S, Fong KM, Maitra A, *et al.* 5′ CpG island methylation of the *FHIT* gene is correlated with loss of gene expression in lung and breast cancer. Cancer Res 2001;61:3581–3585.
26 Noguchi T, Takeno S, Kimura Y, *et al. FHIT* expression and hypermethylation in esophageal squamous cell carcinoma. Int J Mol Med 2003;11:441–447.
27 Uehara E, Takeuchi S, Tasaka T, *et al.* Aberrant methylation in promoter-associated CpG islands of multiple genes in therapy-related leukemia. Int J Oncol 2003;23:693–696.
28 Wu Q, Shi H, Suo Z, *et al.* 5′-CpG island methylation of the *FHIT* gene is associated with reduced protein expression and higher clinical stage in cervical carcinomas. Ultrastruct Pathol 2003;27: 417–422.
29 Gutierrez MI, Bhatia K, Barriga F, *et al.* Molecular epidemiology of Burkitt's lymphoma from South America: differences in breakpoint location and Epstein-Barr virus association from tumors in other world regions. Blood 1992;79:3261–3266.
30 Yang Q, Nakamura M, Nakamura Y, *et al.* Two-hit inactivation of *FHIT* by loss of heterozygosity and hypermethylation in breast cancer. Clin Cancer Res 2002;8:2890–2893.
31 Jaffe ES, Harris NL, Stein H. World Health Organization Classification of Tumours. Pathology & Genetics: Tumours of the Haematopoetic and Lymphoid tissues. IARC Press: Lyon, 2001.
32 Kononen J, Bubendorf L, Kallioniemi A, *et al.* Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998;4:844–847.
33 Rossi D, Gaidano G, Gloghini A, *et al.* Frequent aberrant promoter hypermethylation of O6-methylguanine-DNA methyltransferase and death-associated protein kinase genes in immunodeficiency-related lymphomas. Br J Haematol 2003;123:475–478.
34 Yang Q, Yoshimura G, Suzuma T, *et al.* Clinicopathological significance of fragile histidine triad transcription protein expression in breast carcinoma. Clin Cancer Res 2001;7:3869–3873.
35 Greenspan DL, Connolly DC, Wu R, *et al.* Loss of *FHIT* expression in cervical carcinoma cell lines and primary tumors. Cancer Res 1997;57:4692–4698.
36 Ozaki K, Enomoto T, Yoshino K, *et al. fhit* Alterations in endometrial carcinoma and hyperplasia. Int J Cancer 2000;85:306–312.
37 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;88:217–222.
38 Al-Kuraya KS, Narayanappa R, Siraj AK, *et al.* High frequency and strong prognostic relevance of 06MGMT silencing in diffuse large B-cell lymphomas from the middle east. Hum Pathol 2006 in press [Accepted].
39 Pekarsky Y, Zanesi N, Palamarchuk A, *et al. FHIT*: from gene discovery to cancer treatment and prevention. Lancet Oncol 2002;3:748–754.
40 Aparicio A, Eads CA, Leong LA, *et al.* Phase I trial of continuous infusion 5-aza-2′-deoxycytidine. Cancer Chemother Pharmacol 2003;51:231–239.
41 Schwartsmann G, Schunemann H, Gorini CN, *et al.* A phase I trial of cisplatin plus decitabine, a new DNA-hypomethylating agent, in patients with advanced solid tumors and a follow-up early phase II evaluation in patients with inoperable non-small cell lung cancer. Invest New Drugs 2000;18:83–91.
42 Pohlmann P, DiLeone LP, Cancella AI, *et al.* Phase II trial of cisplatin plus decitabine, a new DNA hypomethylating agent, in patients with advanced squamous cell carcinoma of the cervix. Am J Clin Oncol 2002;25:496–501.
43 Roz L, Andriani F, Ferreira CG, *et al.* The apoptotic pathway triggered by the *Fhit* protein in lung cancer cell lines is not affected by Bcl-2 or Bcl-x(L) overexpression. Oncogene 2004;23:9102–9110.
44 Roz L, Gramegna M, Ishii H, *et al.* Restoration of fragile histidine triad (*FHIT*) expression induces apoptosis and suppresses tumorigenicity in lung and cervical cancer cell lines. Proc Natl Acad Sci USA 2002;99: 3615–3620.
45 Semba S, Trapasso F, Fabbri M, *et al. Fhit* modulation of the Akt-survivin pathway in lung cancer cells: *Fhit*-tyrosine 114 (Y114) is essential. Oncogene 2006 [Epub ahead of print].
46 Sauter G, Simon R, Hillan K. Tissue microarrays in drug discovery. Nat Rev Drug Discov 2003;2:962–972.
47 Torhorst J, Bucher C, Kononen J, *et al.* Tissue microarrays for rapid linking of molecular changes to clinical endpoints. Am J Pathol 2001;159:2249–2256.
48 Tapia C, Schraml P, Simon R, *et al.* HER2 analysis in breast cancer: reduced immunoreactivity in FISH non-informative cancer biopsies. Int J Oncol 2004;25: 1551–1557.
49 Mirlacher M, Kasper M, Storz M, *et al.* Influence of slide aging on results of translational research studies using immunohistochemistry. Mod Pathol 2004;17: 1414–1420.