ELSEVIER

European Journal of Cancer 40 (2004) 1581–1588

European Journal of Cancer

www.ejconline.com

Abnormal Fhit protein expression and high frequency of microsatellite instability in sporadic colorectal cancer

Leopoldo Sarli [a,*], Lorena Bottarelli [b], Cinzia Azzoni [b], Nicoletta Campanini [b], Gabriella Di Cola [c], Giovanni Bader [a], Domenico Iusco [a], Carlo Salvemini [a], Giuseppe Caruso [a], Enrico Donadei [a], Silvia Pizzi [b], Tiziana D'Adda [b], Costi Renato [a], Luigi Roncoroni [a], Cesare Bordi [b]

[a] *Department of Surgical Sciences, Section of General Surgical Clinics and Surgical Therapy, Parma University, Medical School, Via Gramsci 14, 43100 Parma, Italy*

[b] *Department of Pathology and Laboratory Medicine, Section of Pathological Anatomy, Parma University, Medical School, Parma, Italy*

[c] *Research Laboratory "Biotech", Parma, Italy*

Received 3 November 2003; received in revised form 13 February 2004; accepted 26 February 2004
Available online 19 May 2004

Abstract

The role of Fhit protein in the oncogenesis of colorectal cancer is still in debate. Recent studies have revealed that reduced Fhit protein expression is associated with a deficiency of the mismatch repair protein. One hundred and twenty unselected patients who underwent curative resection for sporadic colorectal cancer in a three-year period were evaluated for microsatellite instability (MSI) using six microsatellite markers, and for the presence of Fhit and mismatch repair (MMR) proteins (Mlh1 and Msh2) by means of immunostaining. The relations between these markers were analysed. Reduced or absent Fhit expression was noted in 18 out of 118 patients. This altered expression was significantly higher in right-sided cancer ($P = 0.005$), mucinous tumours ($P = 0.005$) and in poorly differentiated histological types ($P = 0.0001$). MSI was found in 22 out of 109 patients, more so in right-sided cancer ($P = 0.0001$), poorly differentiated histology ($P = 0.0001$), and mucinous tumours ($P = 0.0001$). No association was found with TNM stage. MSI was present in 66.7% of tumours with altered Fhit expression and in only 10% of tumours with preserved or intermediate Fhit expression ($P = 0.0001$). Of the tumours with reduced or absent Fhit expression, 72.2% had loss of nuclear Mlh1 or Msh2 expression compared with only 14% of the preserved or intermediate Fhit expression tumours ($P = 0.0001$). These results support the hypothesis that deficiency in a MMR gene could be a cause of the high frequency of alterations in Fhit expression, and they permit the suggestion that *FHIT* gene alteration may be part of the genetic pathway involving MSI through which some colorectal cancers arise.

Keywords: FHIT; MSI; Colorectal cancer; hMLH1; hMSH2

1. Introduction

The short arm of chromosome 3 has been an important topic for many investigators as a potential tumour-suppressor gene site [1,2]. Recently, the *FHIT* (fragile histidine triad) gene has been cloned and mapped at 3p14.2 [3]. The *FHIT* gene is located at the FRA3B site, and is an example of a relatively well-studied gene located in a constitutively fragile region [3].

Fragile sites are chromosomal structures that have been proposed as having a determining role in cancer-associated chromosomal instability and may affect the function of genes encoded in those chromosome regions. Common fragile sites are FRA3B and FRA16D, which have been found to be the most frequently expressed of the more than 80 common chromosomal fragile sites described. Allelic losses and chromosomal fragility affect the chromosome 16 region spanned by *WWOX*, which

* Corresponding author. Fax: +39-0521-940-125.
E-mail address: leosarli@unipr.it (L. Sarli).

0959-8049/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2004.02.021

behaves as a potent suppressor of tumour growth, suggesting that abnormalities affecting this gene at the genomic and transcriptional level may be of relevance in carcinogenesis [4]. Another common fragile site, *FRA7G*, has also been shown to be located within an approximately 1 Mb region of frequent deletion in breast and prostate cancer [5], as well as squamous cell carcinomas of the head and neck, renal cell carcinomas, ovarian adenocarcinomas and colon carcinomas.

Numerous recent studies have strengthened the position of the *FHIT* gene as a tumour-suppressor gene, including the demonstration of inhibition of tumourigenicity in nude mice by exogenous Fhit protein expression [6] and the induction of apoptosis in cancer cells by over-expression of exogenous Fhit [7–9].

The *FHIT* gene is composed of 10 exons that span a 1.8 Mb genome region, of which only exons 5–9 are protein coding. Fhit protein expression is detected in epithelial cells in most human and mouse tissues [3,10]. In higher eukaryotes, Fhit may reduce the intracellular content of diadenosine triphosphate by binding to it and inducing its hydrolysis [11]. A role of diadenosine triphosphate in the growth control of cells has been suggested [11]. Abnormalities in the *FHIT* gene and/or its expression have been identified in a variety of human cancer cell lines and tumour tissues, including lung [12], breast [13], head-and-neck [14], oesophageal [15], gastric [16], pancreatic [17], renal [18], and cervical [19] cancer. The clinicopathological significance of *FHIT* gene alterations in colorectal cancer has yet to be clearly elucidated. Ohta *et al.* [3] report the presence of initially aberrant transcripts of the *FHIT* gene in three out of eight (38%) colorectal cancer cases. In addition, several reports have described the important role that the *FHIT* gene has in the oncogenesis or the progression of colorectal cancer [20–22]. In contrast, Thiagalingam *et al.* [23] concluded that there is no evidence that the *FHIT* gene is involved in colorectal carcinogenesis.

Recent data have revealed that reduced Fhit protein expression is associated with mismatch repair (MMR) deficiency in human advanced colorectal carcinoma [24].

In this study, we examined the immunohistochemical expression of Fhit, Msh2, and Mlh1 in 120 colorectal cancers, and we analysed the relation of our results to clinicopathological data.

2. Patients and methods

2.1. Study population

The study population consisted of 133 unselected consecutive patients who had undergone curative colorectal resection for sporadic colorectal cancer between July 1996 and April 1999. All cases were deemed sporadic, based on the absence of relevant family history as recorded prospectively at initial patient interview.

A curative operation was defined as one in which no macroscopic tumour remained at the end of surgery and in which histopathological examination of the operative specimen showed no tumour at the margins of resection. Distant metastases at the time of resection were excluded by preoperative liver ultrasonography, chest radiographs and intraoperative exploration. During the study period, a uniform surgical management protocol was in place.

13 patients were excluded on the basis of insufficient tissue for analysis, missing sections or lack of correlation between histology and section available, leaving 120 patients for study. The ages of the 63 male and 57 female patients ranged from 33 to 94 years (mean ± SD: 71.1 ± 11.99 years). In accordance with the classification of tumours by the World Health Organization [25], we defined 50 tumours as mucinous since 50% or more of the tumour displayed the specific cell type; the other 70 tumours were classified as 'adenocarcinoma, not otherwise specified'. Seventy-seven tumours were described as well- or moderately differentiated, and 43 as poorly differentiated. Fifty cases involved right-sided tumours and 70 cases involved left-sided tumours.

Right-sided lesions were defined as those confined to the caecum or ascending or transverse colon; left-sided lesions were defined as those confined to the descending colon, sigmoid colon or rectum.

Tumours were staged in accordance with the TNM system [26]: 11 were at stage I, 58 at stage II, 31 at stage III and 20 at stage IV.

All specimens underwent histopathological review by a single gastrointestinal pathologist (B.C.), who was unaware of the results of molecular genetic and immunohistochemical analysis.

Ethical approval for the study was obtained from the human ethics committee of the University of Parma.

2.2. DNA preparation, loss of heterozygosity and microsatellite instability testing

These evaluations had been done previously, for a study whose results have not yet been published.

Specimens of freshly resected colorectal carcinomas were snap-frozen in liquid nitrogen and subsequently stored at −80 °C. In all cases, fresh specimens of normal colon mucosa were also collected and used as matching controls.

Only tumour samples containing at least 80% of neoplastic cells were included in the study. To verify this condition, one 10 μm-thick cryostat section from each tumour sample was stained with haematoxylin and microscopically examined by a pathologist. Fifteen to 25 cryostat sections (20 μm-thick) from the tumours included in the study and from matching normal samples

were submitted to DNA extraction by QIAGEN DNeasy tissue kit (Qiagen, Hilden, Germany).

2.3. Polymerase chain reaction (PCR)

A panel of six polymorphic microsatellite markers located on chromosomal regions potentially involved in colon carcinoma development and progression was utilised, comprising D18S58 (18q22-23), D18S61 (18q22) [27], BAT26 (2p16), BAT40 (1p13) [28], D8S254 (8p22) [29] and D4S2397(4p14-16) [30].

Primer sequences and amplification conditions were in accordance with the Genome Database information (http://www.gdb.org).

The target sequences in normal and tumour DNA were amplified by PCR in a 25 μl reaction mixture containing 2 μl DNA sample, 10× buffer (10 mM Tris–HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (Promega, Madison, WI), 0.4 μM of each primer and 1.25 U *Taq* polymerase (Promega, Madison, WI). Each forward primer was coupled with the fluorescent dye TET (4,7,2′,7′-tetrachloro-6-carboxyfluorescein) (Applied Biosystems, Foster City, CA). Microsatellites were submitted to 35–40 cycles of amplification in a PTC-100™ thermal cycler (MJ Research Inc., Waltham, MA) at different annealing temperatures: 61 °C for D18S58, 55 °C for D18S61 and BAT40, 49 °C for BAT26, and 57 °C for D8S254 and D4S2397. The presence and correct size of amplimers were evaluated by 2% agarose gel electrophoresis.

2.4. Fragment analysis

Amplimers were separated in a 6% polyacrylamide denaturing gel (Long Ranger, BMA, Rockland, ME) using the GenomyxLR™ DNA sequencer (Beckman Coulter, Fullerton, CA). A portion (1.5 μl) of each PCR product was mixed with 1.5 l of blue dextran loading solution (Promega). After 5 min of denaturation at 95 °C, 2 μl of the mix were loaded on to the gel and subsequently run for 2 h at 2750 V, 125 W and 50 °C. At the end of the electrophoretic run, the fluorescent signals were automatically collected by a GenomyxSC™ scanner and further analysed with the *Clarity*SC™ image-analysis software (Beckman Coulter), which generates electropheretograms in which alleles appear as peaks. Height and area of the peaks calculated by the software are proportional to the concentration of the alleles in the sample.

2.5. Loss of heterozygosity (LOH)

The presence of two alleles in normal tissue was the necessary condition for evaluation of allelic losses.

The following ratio was calculated on the basis of peak-area data: $(\text{lower allele/higher allele})_{\text{tum}}/(\text{lower allele/higher allele})_{\text{norm}}$. Values $\leqslant 0.6$ (allelic imbalance $\geqslant 40\%$) were considered as indicative of LOH.

Cases in which the normal DNA sample was homozygous were classified as non-informative. The ratio of allele peak areas calculated for each tumour sample was divided by the allele peak-area ratio of the normal matching control and, if above 1.00, converted to give a result ranging from 0.00 to 1.00. Ratios below 0.6, indicating at least a 40% reduction of a tumour allele, were indicative of an allelic loss [31].

2.6. Microsatellite instability (MSI)

The novel appearance in the tumour DNA of one or more alleles, i.e. new peaks in the electrophoretogram, not present in its paired normal DNA, was the indicator of MSI [32].

Tumours were classified as demonstrating high-frequency MSI (MSI-H) when instability was detected in at least 30% of the interpretable microsatellite markers investigated, or as low-frequency MSI (MSI-L) when instability was found in less than 30% of the markers, in accordance with international criteria [33]. Tumours without MSI were defined as microsatellite stable (MSS) [33]. For the purposes of this study, MSS and MSI-L cases were considered together [32].

2.7. Immunohistochemical staining

The specimens containing tumour and normal glands were routinely fixed in buffered formalin and embedded in paraffin. Sections (5 μm) were stained with haematoxylin and eosin for histological diagnosis and with the following primary monoclonal antibodies: anti-hmsh2 (Clone FE11; Oncogene Research Products, Cambridge, Massachusetts, USA; working dilution: 1/20); anti-hmlh1 (clone G168-728; Pharmingen, San Diego, California, USA working dilution: 1/75) and with the rabbit anti-Fhit clone (Polyclonal-ZR44; Zymed Laboratories, San Francisco, California, USA, working dilution: 1/50). Antibody hmlh1 recognises the full-length recombinant human hmlh1 protein (83 kDa), and hmsh2 antibody (clone FE11) reacts with the carboxyterminal region of the hmsh2 protein. Anti-Fhit antiserum detects full length (≈17 kDa) Fhit protein in cell and tissue lysates derived from man and rat.

The antibodies, clones, sources, pretreatments, working dilutions, incubation time and localisation of the immunostaining are listed in Table 1.

For antigen retrieval, sections were treated with 10 mM citrate at pH 6.0 in a 750 W microwave oven for three 5-min cycles. The sections were immunostained with a streptavidin–biotin kit (LSAB2; Dako) in accordance with the manufacturer's specifications and counterstained with haematoxylin. Positive internal controls were the normal glands and the intestinal

Table 1
Antibodies, clones, pretreatments, working dilutions, incubation time and localisation of the immunostaining

Antibodies	Clone	Pretreatment	Dilution	Staining	Incubation
hMLH1	G168-728	Citrate buffer, MW	1:75	Nuclei	Overnight
hMSH2	FE11	Citrate buffer, MW	1:20	Nuclei	Overnight
FHIT	Pab- ZR744	Citrate buffer, MW	1:50	Cytoplasm	Overnight

MW, microwave oven.

crypts. Negative controls consisted of substituting normal mouse serum for the primary antibodies.

2.8. Semiquantitative analysis

Immunostaining for hmsh2 (nuclear) and hmlh1 (nuclear) and Fhit (cytoplasmic) was estimated on a semiquantitative score according to the number of positive tumour cells as follows: 0% (0), <10% (1), 10–50% (2), 51–80% (3), or >80% (4). The intensity of staining was also evaluated as weak (1+), moderate (2+) or strong (3+). For each tumour case, the values for the two variables were multiplied, resulting in scores ranging from 0 to 12 [34]. For the purposes of the study, staining of tumour nuclei for hmlh1 and hmsh2, and cytoplasm for Fhit, was evaluated as absent (no protein) or present (any evidence). The 0–6 scores were considered as altered expression, and 7–12 as preserved expression.

In addition, among tumours with preserved gene expression, two groups were distinguished, with low (<50% of positive cells, or scores of 1–6) and high (>50%, or scores of 8–12) expression, respectively.

For Fhit, cytoplasmic staining scores of 9–12 were defined as a strong staining pattern, since there was little difference compared with normal mucosa; scores of 6–8 were defined as an intermediate or heterogeneous staining pattern, and scores of 0–5 were defined as markedly reduced or lost expression [35]. In addition, the scores of 9–12 and 6–8 were analysed together, since they signify preserved gene expression.

2.9. Statistical analysis

Contingency tables and the χ^2 test were used to evaluate differences between percentages.

The significance level was set at $P = 0.05$ and confidence intervals were set at 95%.

3. Results

3.1. Fhit expression

On immunohistochemical staining, all the normal colonic epithelia showed strong cytoplasmic expression of the Fhit protein from the basal cells to the luminal differentiated cells; these served as internal controls. Smooth muscle cells and inflammatory mononuclear cells were positive with varying intensity and extension.

In two cases, the immunohistochemical staining of colorectal cancer cells could not be evaluated. Reduced or absent Fhit expression was noted in 18 of the remaining 118 (15.3%) colorectal cancer cases, preserved in 81 (68.6%) colorectal cancers, and intermediate in 19 (16.1%).

When the relation between these results and the clinicopathological data (age and sex of patients, tumour location, histological type, grading, stage) was analysed, decreased or absent staining for Fhit was detected in 26% of right-sided lesions vs. 7% of left-sided lesions ($P = 0.005$), in 26% of mucinous tumours vs. 7% of not otherwise specified adenocarcinomas ($P = 0.005$), and in 38% of poorly differentiated cancers vs. 3% of well- or moderately differentiated cancers ($P = 0.0001$) (Table 2). No significant associations were found between the Fhit expression and other clinicopathological variables (Table 2).

3.2. Correlation of FHIT expression with MSI and MMR gene expression

Of the 109 tumour specimens evaluated for MSI, 22 (20%) showed MSI-H. MSI-L was found in three (3%) out of 109 cancers, which were categorised as MSS tumours. MSI-H was more likely to be displayed by right-sided tumours ($P = 0.0001$), by poorly differentiated tumours ($P = 0.0001$), by mucinous aspect tumours ($P = 0.0001$) (Table 2), and in female patients ($P = 0.031$). No association was found between microsatellite status and the TNM stage of tumours (Table 2). Among the tumours with reduced or absent Fhit expression, 66.7% (12 out of 18) had MSI compared with only 10% (10 out of 100) of the preserved or intermediate Fhit expression tumours ($P = 0.000$).

The immunohistochemical expression of Mlh1 by colorectal cancer cells could not be evaluated in four cases, and that of Msh2 in four cases. Of the 116 carcinomas, 28 (24.1%) had reduced expression of Mlh1 protein, whereas 12 (10.3%) had reduced expression of Msh2 protein. Reduced expression of both proteins was observed in 10 of the 114 (8.3%) specimens in which it was possible to evaluate the expression of both genes (Table 3). In addition, loss of nuclear Mlh1 or Msh2

Table 2
Relation between Fhit, microsatellite instability (MSI), and mismatch repair (MMR) protein expression and clinicopathological findings in patients with colorectal carcinoma

	Fhit protein expression (118 tumours)			Mlh1/Msh2[b] Expression (114 tumours)			MSI (109 tumours)		
	Preserved[a]	Reduced or absent[a]	*P*	+	–	*P*	+	–	*P*
Sex									
Male	55	7		49	10		7	50	
Female	45	11	0.208	34	21	0.011	15	37	0.031
Tumour localisation									
Right-sided	37	13		27	22		17	28	
Left-sided	63	5	0.005	56	9	0.000	5	59	0.0001
Histological type									
Mucinous	37	13		30	19		17	28	
ADK	63	5	0.005	53	12	0.016	5	59	0.0001
Grading									
Good-moderate	74	2		66	9		4	64	
Poor	26	16	0.0001	17	22	0.0001	18	23	0.0001
TNM stage									
I	7	3		8	2		2	8	
II	49	8		34	20		15	40	
III	28	3		27	3		2	24	
IV	16	4	0 417	14	5	0.059	3	15	0.224

[a] Preserved: score 12–6; reduced or absent: score 5–0.
[b] Presence (+) or absence (–) of either one or both Mlh1 and Msh2.

Table 3
Relation between Fhit and MMR protein expression, and MSI in 103 cases of colorectal carcinoma in which it was possible to evaluate all three variables

	n	MSI		Fhit expression	
		+	–	Preserved intermediate	Reduced or absent
Mlh1 (+), Msh2 (+)	75	2	73	70	5
Mlh1 (–), Msh2 (+)	18	13	5	11	7
Mlh1 (+), Msh2 (–)	1	1	0	0	1
Mlh1 (–), Msh2 (–)	9	6	3	5	4

Reduced Fhit expression is associated with loss of MMR expression: $P = 0.0001$. Reduced Fhit expression is associated with MSI: $P = 0.0001$. Loss of MMR expression is associated with MSI: $P = 0.0001$.

expression was more frequently associated with poor differentiation ($P = 0.0001$), mucinous histology ($P = 0.0001$) and right-sided location ($P = 0.0001$) (Table 2). However, there was no significant difference in other clinicopathological variables (Table 2). Among the tumours with reduced or absent Fhit expression, 72.2% (13 out of 18) had loss of nuclear Mlh1 or Msh2 expression compared with only 14.2% (16 out of 112) of the preserved or intermediate Fhit expression tumours ($P = 0.0001$) (Table 3).

4. Discussion

The results of several studies [19,21] have indicated that *FHIT* gene alterations can be simply but reliably detected by immunohistochemical analysis of tumour specimens. In this study, we observed markedly reduced expression of Fhit protein in 15% of colorectal cancers, a frequency lower than that observed by others [21,22,24]. Significant differences in the frequency of abnormal Fhit expression have been reported in the recent literature [21,22,24,36]. Differences in patient selection procedures may account for these discordant results. In our study, patients affected by sporadic colorectal cancer were selected on the basis of the absence of relevant family history. In contrast, in the study by Andachi *et al.* [24], family history was not an exclusion criterion, and some cases of hereditary non-polyposis colorectal cancer (HNPCC) may have been included in the study population. A higher frequency of Fhit protein expression loss or reduction in HNPCC compared to sporadic cases has been observed [37]. In the study by Hao *et al.* [21], patients with familiar adenomatosis

polypi were included. The distribution of clinicopathological factors (tumour location, histological differentiation, stage) differed in the various studies. On the other hand, the frequency of abnormal MMR protein expression in the study of Andachi *et al*. [24] was higher than that usually reported in the literature [38].

The results of the present study, in agreement with those of recent publications [21,22,24], suggest that loss of *FHIT* gene expression is associated with decreasing degrees of differentiation in colorectal cancers. Similar losses of *FHIT* function have been associated with grade in lung cancer [12]. Markedly reduced *FHIT* expression was significantly more frequent in the right-sided colorectal cancers and in mucinous tumours. This correlation has not been noted in any of the studies that up to now have evaluated clinicopathological features and *FHIT* gene expression in sporadic colorectal adenocarcinoma. In contrast, we found no correlation between *FHIT* gene expression and the clinicopathological stage of the tumour.

Recently, differences have been revealed in molecular genetic features of cancer cells of the large bowel, leading to the hypothesis that colorectal cancer arises through at least two distinct genetic pathways, one involving MSI and the other involving chromosomal instability [39]. Microsatellites are distributed throughout the genome, predominantly in non-coding regions. When MSI affects the coding region of genes critical for cell physiology, it may lead to loss of gene function, thus contributing to malignant transformation [40]. This genetic pathway has recently been described in hereditary tumours, where MSI appears to be a good marker for defects of the MMR genes, but it has also been suggested in sporadic cancer [41]. High-frequency MSI occurs in approximately 15% of sporadic cases of colorectal cancers [41,42]. In the present study, high MSI was detected in 20% of the colorectal cancers studied. The presence of high MSI was closely associated with reduced Fhit expression ($P = 0.0001$).

In accordance with previous studies [41,42], colorectal cancers with MSI were more frequent in right-sided, mucinous adenocarcinomas and in poorly differentiated tumours. The correlation between high tumour MSI and reduced expression of Fhit protein may account for the connections between the clinicopathological features of tumours with MSI and those with reduced expression of Fhit.

Among the known MMR genes, germline mutations of hMLH1 (3p21–23) and hMSH2 (2p21–22) have been predominantly associated with MSI in the Lynch II HNPCC syndrome, but also in sporadic colorectal cancer [43]. Previous studies have demonstrated that immunohistochemical analysis of Mlh1 and Msh2 expression could be an accurate and rapid screening procedure for the identification of MMR gene alterations [44,45]. As confirmation of this, in the present study, MMR deficiency based on the status of Mlh1 and Mlh2 protein expression was significantly associated with high MSI. MMR deficiency was detected in 19 out of 22 (86%) colorectal cancers with high MSI. Our data also show that MMR deficiency based on the status of the Mlh1 and Mlh2 protein expression is significantly associated with reduced Fhit protein expression. Interestingly, one out of two cases with MSI and without altered expression of Mlh1 or Msh2 showed markedly reduced Fhit expression, and two out of eight cases with MMR deficiency without MSI showed markedly reduced Fhit expression.

It has previously been observed that human pancreatic cancers and cell lines with high MSI frequently had homozygous deletions of *FHIT* [46]. The Fhit-deficient mouse tumours do not show MSI, and loss of Fhit expression plays a part in their Muir–Torre syndrome (MTS)-like disease; thus, it is unlikely that the mouse syndrome involves MMR deficiency. If human and mouse MTS cases arise through similar mechanisms, then the *FHIT* gene may be a target for damage in a fraction of MMR-deficient tumours, especially those with *MSH2* deficiency, leading to Fhit protein loss and clonal expansion of *Fhit*-negative cells. If *Fhit* inactivation is a frequent result of mismatch repair deficiency, and a frequent pathway to MTS, then *Fhit* +/− mice could be considered as being predisposed to MTS [47].

In conclusion, the results of the present study, in combination with previous reports [48], support the hypothesis that deficiency in an MMR gene could be a cause of the high frequency of altered Fhit expression observed our study, and they allow for the hypothesis that *FHIT* gene alterations may be part of the genetic pathway involving MSI, which is responsible for the development of some colorectal cancers.

Acknowledgements

This work was supported in part by a grant from Lega Italiana per la lotta contro i tumori.

References

1. Kok K, Osinga J, Carritt B, *et al*. Deletion of a DNA sequence at the chromosomal region 3p21 in all major types of lung cancer. *Nature* 1987, **330**, 578–581.
2. Zbar B, Brauch H, Talmadge C, Linehan M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. *Nature* 1987, **327**, 721–724.
3. 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**, 587–597.
4. Bednarek AK, Keck-Waggoner CL, Daniel RL, Laflin KJ, Bergsagel PL, Kiguchi K, *et al*. WWOX, the FRA16D gene, behaves as a suppressor of tumour growth. *Cancer Res* 2001, **61**(22), 8068–8073.

5. Engelman JA, Zhang XL, Lisanti MP. Genes encoding human caveolin-1 and -2 are co-localized to the *D7S522* locus (7q31.1), a known fragile site (FRA7G) that is frequently deleted in human cancers. *FEBS Lett* 1998, **438**, 403–410.
6. 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.
7. Ji L, Fang B, Yen N, Fong K, Minna JD, Roth JA. Induction of apoptosis and inhibition of tumorigenicity and tumour growth by adenovirus vector-mediated fragile histidine triad (FHIT) gene overexpression. *Cancer Res* 1999, **59**, 3333–3339.
8. Sevignani C, Calin GA, Cesari R, Sarti M, Ishii H, Yendamuri S, *et al*. Restoration of fragile histidine triad (FHIT) expression induces apoptosis and suppresses tumorigenicity in breast cancer cell lines. *Cancer Res* 2003, **63**(6), 1183–1187.
9. 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 USA* 2002, **99**(6), 3615–3620, Epub 2002 Mar 12.
10. Druck T, Hadaczek P, Fu TB, *et al*. Structure and expression of the human FHIT gene in normal and tumour cells. *Cancer Res* 1997, **57**, 504–512.
11. Barnes LD, Garrison PN, Siprashvili Z, *et al*. Fhit, a putative tumour suppresser in humans, is a dinucleoside 5′,5′-P1,P3-triphosphate hydrolase. *Biochemistry* 1996, **35**, 11529–11535.
12. 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**, 2533–2536.
13. Campiglio M, Pekarsky Y, Menard S, Tagliabue E, Pilotti S, Croce CM. FHIT loss of function in human primary breast cancer correlates with advanced stage of the disease. *Cancer Res* 1999, **59**, 3866–3869.
14. 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.
15. 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 oesophageal squamous cell carcinomas. *Cancer Res* 1998, **58**, 3429–3434.
16. Gemma A, Hagiwara K, Ke Y, *et al*. FHIT mutations in human primary gastric cancer. *Cancer Res* 1997, **57**, 1435–1437.
17. Sorio C, Baron A, Orlandini S. The FHIT gene is expressed in pancreatic ductular cells and is altered in pancreatic cancers. *Cancer Res* 1999, **59**, 1308–1314.
18. Xiao GH, Jin F, Klein-Szanto AJ, *et al*. The FHIT gene product is highly expressed in the cytoplasm of renal tubular epithelium and is down-regulated in kidney cancers. *Am J Pathol* 1997, **15**, 1541–1547.
19. Birrer MJ, Hendricks D, Farley J, *et al*. Abnormal Fhit expression in malignant and premalignant lesions of the cervix. *Cancer Res* 1999, **59**, 5270–5274.
20. Hibi K, Taguchi M, Nakamura H, *et al*. Alternative splicing of the FHIT gene in colorectal cancers. *Jpn J Cancer Res* 1997, **88**, 385–388.
21. 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.
22. 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**, 7379–7382.
23. Thiagalingam S, Lisitsyn NA, Hamaguchi M, *et al*. Evaluation of the FHIT gene in colorectal cancers. *Cancer Res* 1996, **56**, 2936.
24. 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**, 441–445.
25. Jass JR, Sobin LH. Histological typing of intestinal tumours. 2nd ed. Berlin, Springer, 1989.
26. Compton C, Fenoglio-Preiser CM, Pettigrew N, Fielding LP. American Joint Committee on Cancer Prognostic Factors Consensus Conference. Colorectal Working Group. *Cancer* 2000, **88**, 1739–1757.
27. Jen J, Kim H, Piantadosi S, *et al*. Allelic loss of chromosome 18q and prognosis in colorectal cancer. *N Engl J Med* 1994, **331**, 213–221.
28. Halling KC, French AJ, McDonnel SK, *et al*. Microsatellite instability and 8p allelic imbalance in stage B2 and C colorectal cancers. *J Natl Cancer Inst* 1999, **91**, 1295–1303.
29. Samowitz WS, Slattery ML, Potter JD, Leppert MF. BAT-26 and BAT-40 instability in colorectal adenomas and carcinomas and germline polymorphisms. *Am J Pathol* 1999, **154**, 1637–1641.
30. Arribas R, Ribas M, Risques RA, *et al*. Prospective assessment of allelic losses at 4p14-16 in colorectal cancer: two mutational patterns and a locus associated with poorer survival. *Clin Cancer Res* 1999, **5**, 3454–3459.
31. Beckmann MW, Picard F, An HX, *et al*. Clinical impact of detection of loss of heterozygosity of BRCA1 and BRCA2 markers in sporadic breast cancer. *Br J Cancer* 1996, **73**, 1220–1226.
32. Muller JD, Heagle N, Keller G. Loss of heterozygosity and microsatellite instability in de novo versus ex-adenoma carcinomas of the colorectum. *Am J Pathol* 1998, **153**, 1977–1984.
33. Boland CR, Thibodeau SN, Hamilton SR, *et al*. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998, **58**, 5248–5257.
34. Peiró G, Diebold J, Lohse P, *et al*. Microsatellite instability, loss of heterozygosity, and loss of hMLH1 and hMSH2 protein expression in endometrial carcinoma. *Hum Pathol* 2002, **33**, 347–354.
35. Hao XP, Willis JE, Pretlow TG, Rao JS, MacLennan GT, Talbot IC, *et al*. Loss of fragile histidine triad expression in colorectal carcinomas and premalignant lesions. *Cancer Res* 2000, **60**(1), 18–21.
36. Mady HH, Melhem MF. FHIT protein expression and its relation to apoptosis, tumour histologic grade and prognosis in colorectal adenocarcinoma: an immunohistochemical and image analysis study. *Clin Exp Metastasis* 2002, **19**, 351–358.
37. Hadaczek P, Debniak T, Kurzawski G, *et al*. Fhit protein expression in hereditary and sporadic colorectal cancers. *Pol J Pathol* 2001, **52**, 125–132.
38. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996, **87**, 159–170.
39. Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. *Nature* 1997, **386**, 623–627.
40. Schwartz S, Yamamoto H, Navarro M, *et al*. Frameshift mutations at mononucleotide repeats in caspase-5 and other target genes in endometrial and gastrointestinal cancer of the microsatellite mutator phenotype. *Cancer Res* 1999, **59**, 2995–3002.
41. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 1993, **363**, 558–561.
42. Aaltonen LA, Salovaara R, Kristo P, *et al*. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998, **338**, 1481–1487.
43. Liu B, Nicolaides NC, Markowitz S, *et al*. Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nat Genet* 1995, **9**, 48–55.
44. Thibodeau SN, French AJ, Roche PC, *et al*. Altered expression of hMSH2 and h MLH1 in tumours with microsatellite instability and genetic alterations in mismatch repair gene. *Cancer Res* 1996, **56**, 4836–4840.

45. Marcus VA, Madlensky L, Gryfe R, *et al*. Immunohistochemistry for hMLH1 and hMSH2: a practical test for DNA mismatch repair-deficient tumours. *Am J Surg Pathol* 1999, **23**, 1248–1255.
46. Hilgers W, Kern SE. Molecular genetic basis of pancreatic adenocarcinoma. *Genes Chromosomes Cancer* 1999, **26**, 1–12.
47. Fong LY, Fidanza V, Zanesi N, Lock LF, Siracusa LD, Mancini R, *et al*. Muir-Torre-like syndrome in Fhit-deficient mice. *Proc Natl Acad Sci USA* 2000, **97**(9), 4742–4747.
48. Turner BC, Ottey M, Zimonjic DB, Potoczek M, Hauck WW, Pequignot E, *et al*. The fragile histidine triad/common chromosome fragile site 3B locus and repair-deficient cancers. *Cancer Res* 2002, **62**(14), 4054–4060.