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Fhit protein is preferentially expressed in the nucleus of monocyte-derived cells and its possible biological significance

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Summary. The FHIT gene encompassing the most active common human fragile region, FRA3B, has been proposed as a tumour suppressor gene for important common human carcinomas. The mechanism in which Fhit protein exerts its tumour suppressor activity is still obscure. To further understand the Fhit function associated with its intracellular localization we have investigated its cellular localization and distribution in human normal and cancerous tissues. Data of 1500 samples from immunohistochemistry showed that Fhit protein was preferentially and stably expressed in the nucleus of monocyte-derived or histiocytic lineage cells including monocytes of the circulating blood cells, macrophages of the connective tissue, Kupffer cells of the liver, alveolar macrophages or dust cells of the lung, osteoclasts of bone, microglia of the brain, epithelioid cells under chronic inflammatory conditions, foreign-body giant cells, Langerhans cells of the epidermis and dendritic cells of various kinds of human tissue, although the protein could also be infrequently observed in the nucleus of some quiescent epithelial cells. In active cells other than histiocytes, Fhit protein was detected either in cytoplasm or was negative. Neurons expressed Fhit strongly and neuroglial cells did so moderately but only in the cytoplasm. There was no Fhit protein detected in the neutrophils, lymphocytes, plasma cells and lipocytes. The present data showes that the stable nuclear localization of Fhit is not only a special marker for histiocytes with various morphologies but also may suggest the other function concerning Fhit as a signaling molecule related to anti-proliferation function. The detailed biological function related to nuclear localization of Fhit protein in the histiocytes remains to be further studied.

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Introduction

The fragile histidine triad gene (FHIT) located at chromosome 3p14.2(Ohta et al., 1996; Heubner et al., 1998; Heubner and Croce, 2002) is altered in many kinds of primary or cultured carcinomas in the form of deletion within both FHIT alleles, resulting in loss of exons and concomitant absence of full-length FHIT transcript and protein (Siprashvili et al., 1997; Croce et al., 1999). Reversion of tumourigenicity was observed when FHIT gene transfected into lung cancer cell line lacking Fhit protein expression by a significant inhibition of cell growth, in which the apoptotic rate was greatly raised by an up-regulation of p21waf transcripts and Bak protein (Sard et al., 1997; Heubner, 2003). It is suggested that the FHIT tumour-suppressor function is related to induction of apoptosis and cell cycle alteration. Golebiowski et al (Golebiowski et al., 2001) first reported the distribution of Fhit protein in rat tissues and its intracellular localization from subcellular fractions of various tissues by different centrifugation and density-gradient centrifugation, in which they found that Fhit protein was only located in the nucleus and the plasma membrane in rat cells. It was contradictory to many reports by immunohistochemistry, indicating Fhit protein was located in the cytoplasm of non-neoplastic and cancerous tissues. To elucidate the intracellular localization of Fhit protein in human normal or non-neoplastic tissues and cancerous tissues, we investigated a large sample of carcinomatous tissues and their normal or non-neoplastic counterparts by immunohistochemistry and explored its possible biological significance.

Materials and methods

Up to 1500 biopsy samples of human beings

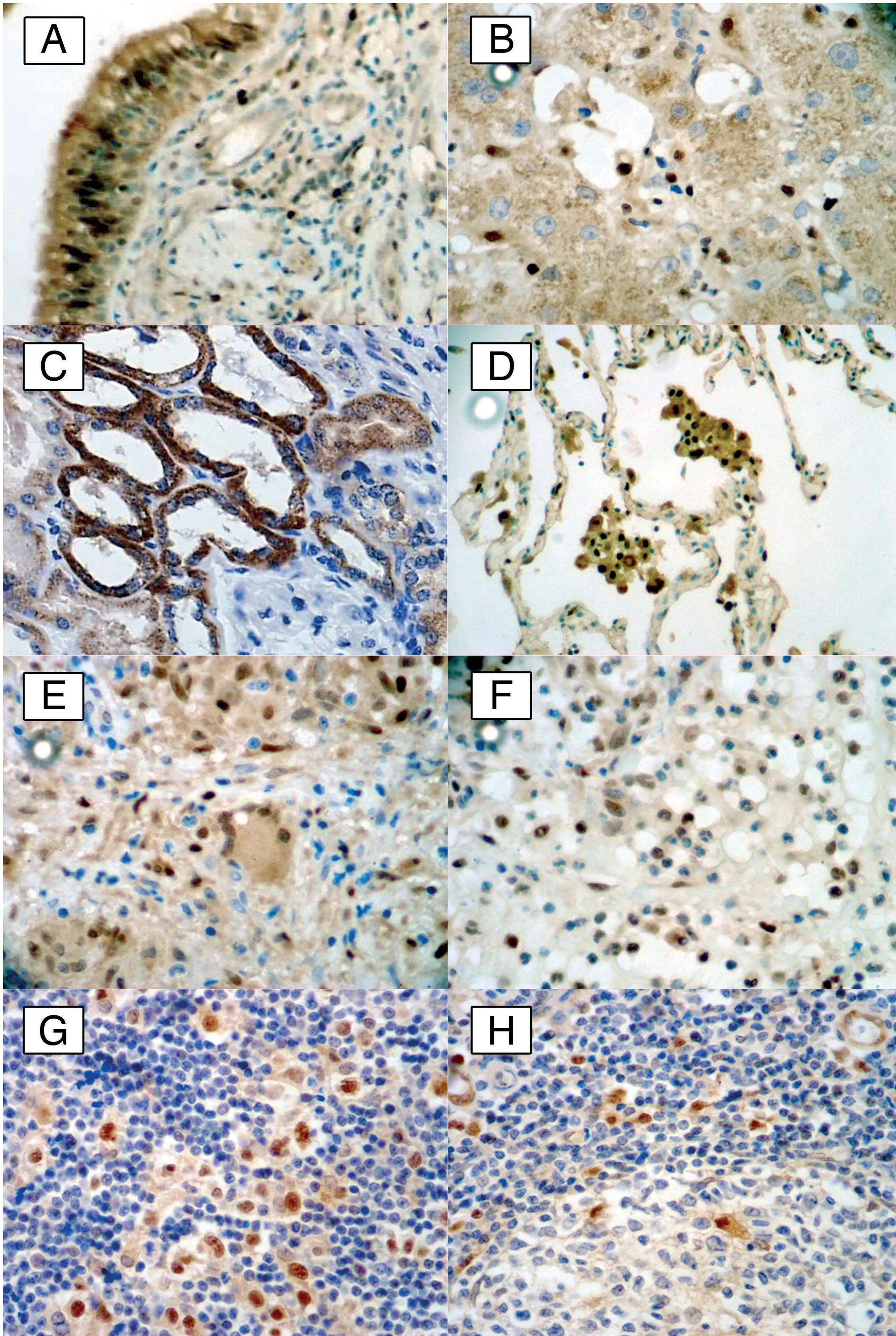
including 893 cancerous and 607 normal or non-neoplastic tissues were analyzed in this study. All samples of patients were from the largest hospital in China, Chinese People's Liberation Army (PLA) General Hospital, Beijing and were formalin-fixed, paraffin-embedded in wax blocks. The carcinomas were graded and staged by World Health Organization (WHO) criteria. Immunocytochemical staining for FHIT protein (Fhit) was performed by a standard two-step method except that the pressure-cooking procedure was used for antigen retrieval pretreatment (Hao et al., 2000). Sections were cut 4 µm thick and dewaxed in xylene and rehydrated in a graded ethanol series. Then sections were immersed in 3% hydrogen peroxide in methanol for 10 minutes to block endogenous peroxidase activity and rinsed in running water. Slides were immersed in boiling 0.01 M citrate buffer (pH 6.0) in a pressure cooker and the pressure cooker was then sealed and brought to full pressure. The heating time was 2 minutes, beginning only when full pressure was reached. At 2 minutes the cooker was depressured and cooled under running water. The lid was then removed, and the hot buffer was flushed out with cold water from a running tap. The cooled sections were washed twice in PBS before immunocytochemical staining. The primary polyclonal rabbit antibody against human FHIT protein (Zymed, USA) was diluted 1 in 100 with 0.01 M PBS (pH 7.2) and added to the tissue on the sections. After exposure to primary antibody for one hour the sections were allowed to react with the ready-to-use reagents of poly peroxidase-anti-mouse/rabbit IgG for 20 minutes by the standard PV-6000 Polymer Detection System For Immuno-Histological Staining (GBI, USA), which is the non-biotin HRP detection system to avoid any possible endogenous biotin by heating. A previously known positive human spleen tissue was used as a positive control. The primary antibody was replaced by 0.01 M PBS, as a negative control. The positivity of staining was then scored according to the previous report (Hao et al., 2000). SPSS 10.0 for Windows, Fisher's exact test and Pearson's $\chi 2$ test with continuity correction for trends in proportions were used to assess the associations between FHIT expression and pathological data. $P<0.05$ was considered significant.

Results

The results in this study (Table 1) showed that on average 61.9% (500/808) of 11 kinds of most common carcinomas in Chinese cancer patients showed absent or markedly reduced expression of FHIT protein in cancer cells. The highest loss of FHIT expression is found in small cell lung cancer (82.5%) and renal cell carcinoma (81.8%). A similar reduction of FHIT protein expression has been reported in human carcinomas by researchers in Western industrial world and Japan (Heubner et al., 2003). All common cancers in our study showed higher than 50%, but prostate cancer, which is infrequent in China, was only 12.7% (7/55), for loss of FHIT protein expression (Table 1). The significance was overwhelmingly found in the difference of FHIT expression between cancer tissue and normal or noncancerous tissue, in which all common carcinomas in China were associated with the loss of expression of FHIT ($P<0.0001$) but by contrast prostate carcinoma and prostatic intraepithelial neoplasia (PIN) were corresponded to the overexpression of the protein ($P<0.0001$). The latter is of great interest as prostate cancer is the most common cancer killer in Western male patients, but a definitely infrequent tumour in native

Table 1. FHIT loss in Chinese cancer patients.

Group	FHIT Reactivity			FHIT Negative rate
	Number	Positive	Negative	(%)
Total samples	1500	908	592	39.5
Cancerous	893	356	537	60.1
Normal or noncancerous	607	552	55	9.1
Lung Cancer	151	69	82	54.3
Squamous carcinoma	35	11	24	68.6
Adenocarcinoma	17	13	4	23.5
Neuroendocrine cancer	59	38	21	35.6
Small cell carcinoma	40	7	33	82.5
Normal lung	10	10	0	0
Esophageal Cancer	53	23	30	57.6
Normal esophagus	10	10	0	0
Gastric Cancer	76	28	48	63.2
Dysplastic mucosa	58	22	36	62.1
Normal stomach	10	10	0	0
Colorectal Cancer	60	27	33	55.0
Colorectal adenoma	20	20	0	0
Normal large intestine	10	10	0	0
Liver Cancer	83	33	50	60.2
Para-neoplastic liver	83	83	0	0
Normal liver	10	10	0	0
Pancreatic Cancer	50	22	28	56.0
Normal pancreas	50	50	0	0
Bladder Cancer	51	20	31	60.8
Para-neoplastic bladder	51	48	3	5.9
Normal bladder	10	10	0	0
Renal Cell Cancer	55	10	45	81.8
Para-neoplastic kidney	52	52	0	0
Normal kidney	10	10	0	0
Cervical Cancer	100	32	68	68.0
Paraneoplastic cervix	83	65	18	21.9
Normal cervix	10	10	0	0
Ovarian Cancer	34	16	18	52.9
Ovarian adenoma	14	12	2	14.3
Normal ovary	10	10	0	0
Breast Cancer	66	28	38	57.6
Normal breast	66	66	0	0
Prostate Cancer	55	48	7	12.7
Prostatic intraepithelial Neoplasia (PIN)	42	9	33	78.6
Normal prostate	43	7	36	83.7
Lymphoma	30	0	30	100.0
B cell lymphoma	20	0	20	100.0
T cell lymphoma	10	0	10	100.0
Normal or non-neoplastic Lymph nodes	10	0	10	100.0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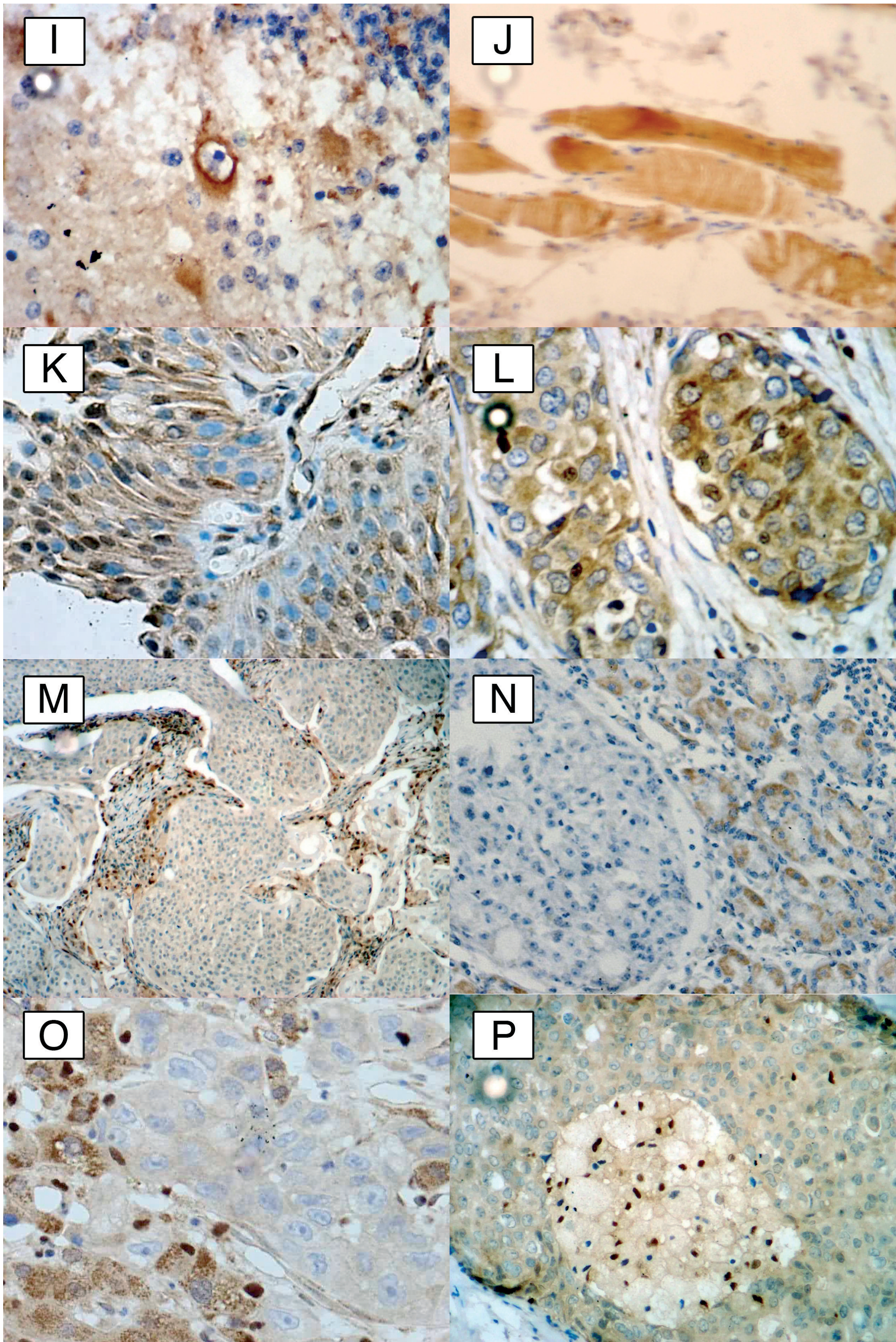
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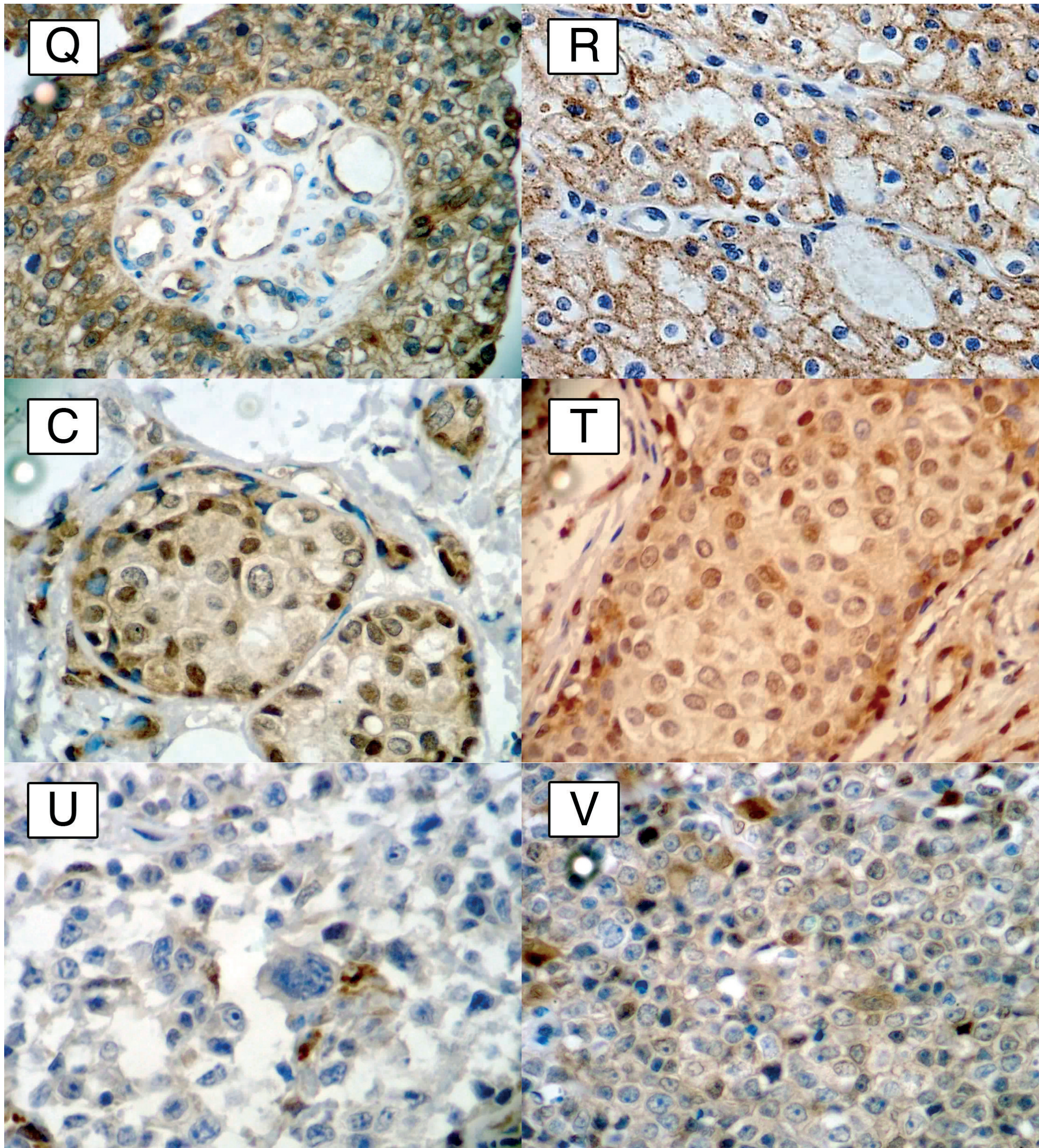

Fig. 1. Immunocytochemical analysis of Fhit expression in human tissues. **A.** Fhit was detected in the upper cells of bronchial epithelia. **B.** Fhit protein was expressed strongly in the nucleus of Kupffer cells or sinusoid macrophages and moderately in the cytoplasm of hepatocytes. **C.** Tubular epithelia were positive but glomeruli of kidney negative for Fhit. **D.** Fhit expression strongly in the nucleus with weakly in the cytoplasm of alveolar macrophages or dust cells. **E.** Fhit was positive in histiocyte aggregating formed granuloma and giant cells. **F.** Neutrophils with multilobed nucleus were negative, contrasted to histiocytes with kidney-like nucleus positive for Fhit. **G.** Lymphocytes in T region of lymph node were negative, whereas histiocytes were strongly positive for Fhit. **H.** Lymphocytes in B region of lymph node were also negative for Fhit. **I.** Fhit protein was also expressed strongly but only in the cytoplasm of neurons and weakly in neuroglial cells **J.** Fhit expression in skeletal muscle. **K.** Upper and slender cells were positive but basal cells negative for Fhit in bladder papillary tumour. **L.** Macrophage with nuclear staining Fhit was phagocytosing (arrow) in hepatocellular carcinoma tissue, in which cancer cells were positive for Fhit only in the cytoplasm. **M.** No Fhit expression in the lung squamous carcinoma cells, except around the cancer nests there were a lot of Fhit-positive histiocytes. **N.** Fhit protein was not detected in gastric cancer cells (left). **O.** Fhit was not detected in hepatocellular carcinoma cells, histiocytes with strongly nuclear staining were distinctly observed. **P.** In the lumen of breast ductal carcinoma in situ Fhit was expressed in the nucleus of foamy cells formed by macrophages. **Q.** Membranous and cytoplasmic expression of Fhit was observed in transitional cell papillary carcinoma of bladder. **R.** Membranous pattern of Fhit expression in renal clear cell carcinoma. **S and T.** Fhit expression in the nuclei of smaller tumour cells but not in bigger cancer cells in breast intraductal carcinoma. **U and V.** T and B lymphoma cells were negative for Fhit as well.

Chinese. It should be further studied in detail later.

In epithelial tissue, Fhit protein was found mainly located in the cytoplasm of both normal and non-neoplastic epithelial cells. From base to top, unstable Fhit protein could be gradually present in the nucleus of epithelial cells sometimes, and usually the cells looked slightly atrophied, with smaller and slender cell or nuclear size and with little or no nucleolus. In contrast, the cells with Fhit protein only in the cytoplasm looked more active, usually with bigger cellular and nuclear size, vesicular nucleus and prominent nucleolus. Some cells with the same active morphology might be negative, expressing Fhit protein neither in the nucleus nor in the cytoplasm at all events (Fig. 1A-C).

In connective tissue, the Fhit protein could be found in the histiocytes, fibroblasts and myofibroblasts. In the histiocytes including macrophage and dendritic cell, the strongly expressed Fhit protein was always located in the nucleus, although the Fhit could also be found in the cytoplasm in a few active cells with bigger cellular and nuclear size (Fig. 1B, D, E, F); the same situation was also observed in the monocytes located in the blood vessels. In the fibroblasts, the Fhit could usually be detected in the quiescent cells with slender nucleus but not in the active ones with round or ovoid nucleus and nucleolus. There was no Fhit protein detected in the neutrophils, lymphocytes (Fig. 1F, G, H), plasma cells and lipocytes. Fhit protein could also be detected infrequently and weakly in the nucleus and cytoplasm of endoepithelial cells.

In brain tissue Fhit protein was strongly expressed in the nucleus of microglia, which was also the monocyte-derived cells. Fhit protein was expressed strongly but only in the cytoplasm of neurons and also moderately or weakly in the same part of neuroglial cells (Fig. 1I). In liver tissue the Fhit was distinctively found in the nucleus of Kupffer cells (Fig. 1B), the resident macrophages in the sinusoids, besides the medium expression of Fhit protein could be present in the cytoplasm of hepatocytes. In spleen the most strongly positive cells were also the macrophges in the vicinity of the sinusoids and in the marginal zone. There was no expression of Fhit protein in the kidney glomerulus, whereas the tubular cells expressed the protein in the cytoplasm (Fig. 1C). In muscle, positive staining of Fhit could be observed in skeletal muscle (Fig. 1J) but not in cardiac and smooth muscle.

In cancerous tissue, three expressive patterns of Fhit protein could be found in the carcinoma cells (Fig. 1K-N): up to 60.1% of carcinomas on average showed absent or marked reduced expression of Fhit protein in caner cells (Fig. 1M-P); most positive cancer cells showed cytoplasmic Fhit, usually weaker, but sometimes stronger than, their original or derived cells (Fig. 1L, Q), the result here was associated with other previous studies; the membranous pattern of Fhit could be observed in bladder transitional cell papillary carcinoma and renal clear cell carcinoma (Fig. 1Q, R); a few kinds of cancer cells showed nuclear Fhit staining, particularly in breast intraductal carcinomas with smaller nucleus, similar to their normal counterparts (Fig. 1S, T). Fhit protein was not detectable in B and T type lymphomas (Fig. 1U, V) or their normal counterparts (Fig. 1G, H). Histiocytes including macrophages and dendritic cells or Langerhans cells in cancer tissue always preferentially showed nuclear Fhit localization, sharply contrasting to other kinds of cells that usually presented cytoplasmic or negative pattern of Fhit protein in the histological background (Fig. 1L, O, P). Thus prominent Fhit staining nuclei could easily be observed in the histiocytes within the cancerous tissue, showing various kinds of nuclear contour of cell in morphology, small or big, round or spindle, and irregular or twisted, and spreading between the cancer cells.

Discussion

FHIT gene located at human chromosome region 3p14.2 was identified and shown to be a large gene that encompassed the FRA3B common fragile site, numerous carcinoma cell-specific homozygous and hemizygous deletions, and a familial renal cancer chromosome translocation break (Druck et al., 1998; Huebner et al., 1998). FHIT gene is shown to be about 1 Mb in size and encodes a 1.1-kb cDNA with 10 small exons. FHIT RNA expression was frequently altered by many studies of various common human carcinoma types, and the alterations to RNA expression were also shown to be related to a deletion within the FHIT gene. Furthermore, lack of detectable Fhit protein in both cancer cell lines and solid tumours was found correlated to be with FHIT gene deletions and altered RNA expressions. Alterations in the FHIT gene and/or its expression have been found in primary tumours and cell lines of lung (Fong et al., 1997; Sozzi et al., 1997), breast (Negrini et al., 1996; Bieche et al., 1998; Campiglio et al., 1999), head and neck (Virgilio et al., 1996), esophagus (Michael et al., 1997; Zou et al., 1997; Menin et al., 2000), stomach (Tamura et al., 1997; Baffa et al., 1998; Lee et al., 2001), colon and rectum (Thiagalingam et al., 1996; Hao et al., 2000), pancreas (Sorio et al., 1999), kidney (Hadaczek et al., 1998; Werner et al., 2000), cervix (Greenspan et al., 1997; Yoshino et al., 1998; Birrer et al., 1999), and hepatocellular carcinomas (Chen et al., 1998; Gramantieri et al., 1999; Keck et al., 1999; Schlott et al., 1999; Yuan et al., 2000; Zhao et al., 2003). Therefore FHIT gene is considered as a strong candidate tumour suppressor gene that could play an important role in major human carcinomas, because cancer cell-specific homozygous deletions within a gene and lack of expression of the protein product are hallmarkers of tumour suppressor gene. There were a few reports published in exploring its intracellular localization that related to function but little was known about the antitumour function contributed by its intracellular localization so far. Golebiowski et al. (2001) first reported the results of distribution of Fhit protein and its intracellular localization in rat tissues and cells. The

immunoblot analysis performed on the subcellular fraction of various rat tissues obtained by the differential and density-gradient centrifugation showed that Fhit protein was localized exclusively in the nucleus and the plasma membrane, supporting the hypothesis concerning Fhit as a signaling molecule (Golebiowski et al., 2001), which was corresponded to the hypothesis that the enzyme-substrate complex from membrane to nucleus is the active form of Fhit. The authors also discovered that Fhit protein was most abundant in spleen and brain. However, they did not show the evidence in situ to find out the exact cell type. In this study we found that in human cells, Fhit protein was located in the nucleus and cytoplasm, the latter could be in the plasma membranous system (endoplasmic reticulum, Golgi network, mitochondria and transport vesicles) associated with the results from Golebiowski et al. (2001). Usually in the quiescent cells showing smaller or irregular nucleus with no or only little nucleolus in morphology, Fhit protein was located in the nucleus, whereas in the active cells showing bigger nucleus and prominent nucleolus, the Fhit was found either only in the cytoplasm or totally absent, which corresponded to the anti-proliferation function (Siprashvili et al., 1997; Sard et al., 1999). Actually there is an overlap between the two positively distributed patterns of Fhit in some cells, showing both nuclear and cytoplasmic localization besides the negative one. Most interestingly, Fhit protein is always most prominently found in the nucleus of histiocytes including macrophage and dendritic cells within one observable microscopic background, strongly suggesting it can be used as a useful marker for histiocytes in differential diagnosis, and can also strongly mean its nuclear signal role related to the function of histiocytes. The functions of histiocytes are as following: first, migrating, phagocytosing and destroying dead and defunct cells (such as senescent erythrocytes), as well as antigens and foreign particulate matter (such as bacteria), in which the destruction occurs within the phagosomes both via enzymatic digestion and through the formation of superoxide, hydrogen peroxide, and hypochlorous acid; second, producing cytokines (such as IL-1, IL-2, IL-12 and tumour necrosis factor α) which activate the inflammatory response as well as the proliferation and maturation of other cells; third, phagocytosing antigens and their most antigenic portions, the epitopes, in conjunction with the integral proteins, class II human leukocytes antigen (class II HLA) to immunocompetent cells; fourth, forming foreign-body giant cells that are large enough to phagocytose the foreign particle by fusing with one another in response to large foreign particulate matter. The most distinguishing or characteristic features of histiocytic lineage or monocyte-derived cells are that these cells have cell-surface Fc (antibody) and C3 (complement) receptors, and can phagocytose, process foreign antigens, and further present epitopes of processed foreign antigens to lymphocytes. Thus they are called antigen-presenting cells. We observed the localization of Fhit in the nucleus of histiocytic lineage cells and tried to find the difference between macrophages and follicular dendritic cell (FDC). But actually there was no difference in intensity or distribution of Fhit between them except that cytoplasmic Fhit could also be found in some active macrophages, which might mean the dinucleoside polyphosphate hydrolase activity of Fhit in the cytoplasm (Barnes et al., 1996).

Most antigen-presenting cells are monocyte-derived and thus belong to the mononuclear phagocyte system including macrophage, dendritic cells, Langerhans cells of the epidermis and oral mucosa, except two types of non-monocyte-derived cells (epithelial reticular cells of the thymus and B cells). Stable nuclear Fhit protein is preferentially found in the monocyte-derived cells, perhaps suggesting that the protein is in accordance with MHC molecules. Like T helper cells, antigen-presenting cells also manufacture and release some cytokines required to activate target cells to perform their specific functions not only in the immune response but also in other processes. Golebiowski et al reported that Fhit protein is most abundant in spleen and brain but they could not sort out the responsible cell that expresses the protein most. In our study we found that the very cell is the macrophage in spleen and, the microglia, neuroglial cell and neuron in brain. The results in which Fhit protein could be detected in human skeletal muscle by this research was different from that found in rat by Golebiowski et al. although the result in smooth and cardiac muscle was alike.

Stable nuclear Fhit protein could be prominently found in the monocytes of the circulating blood cells, macrophages of the connective tissue, Kupffer cells of the liver, alveolar macrophages or dust cells of the lung, osteoclasts of bone, microglia of the brain, epithelioid cells under chronic inflammatory conditions, foreign-body giant cells, Langerhans cells of the epidermis and dendritic cells of the lymph node. The neutrophil, another strongly migratory and phagocytic functional cell besides macrophage, did not express Fhit protein at all, either in nucleus or in cytoplasm, contrary to the result in monocyte-derived cells, suggesting Fhit protein in the nucleus may not be a signal molecule mediating migration and phagocytosis but a signal protein for antigen-presenting and /or cytokine-manufacturing function of histiocytes. The executive mode of the nuclear function may be by Fhit protein itself or by binding with other effector molecules that have been reported, such as tubulin (Chaudhuri et al., 1999), Ubc9 (Shi et al., 2000), Nit (Pekrsky et al., 1998), Hint, CDK7 (Korsisaari and Makela, 2000) and so on.

Previous research results have shown that Fhit cytoplasmic expression is enough to induce apoptosis by FHIT gene transfer to Fhit negative cancers (Siprashvili et al., 1997; Sard et al., 1999), although the detailed mechanism is still obscure. The Fhit protein is highly stably expressed in the nucleus of both fixed or resident and free or elicited macrophages, suggesting Fhit protein

is not only a tumour suppressor protein but also a signal molecule related to immune function. The former one may not be working in macrophages since all histiocytes, no matter how quiescent or active, express Fhit stably and strongly in the nucleus. On the other hand it can also explain why the genuine histiocytic tumour is so rare or absent; but the latter may be the major special effector for histiocyte because of the particularly stable nuclear expression pattern. Therefore further investigation should be carried out to clarify its function to downstream gene and effector molecules.

In summary Fhit protein is preferentially and stably expressed in the nucleus of monocyte-derived cells including monocyte, macrophage and dendritic cells, thus it is a distinguished marker for all kinds of monocyte-derived or histiocytic lineage cells. The function of Fhit nuclear localization in the histiocytes is not clear so far but it may not be correlated with migration and phagocytosis as Fhit is not detected in the neutrophils and lymphocytes, and may not be associated necessarily with pro-apoptotic function because Fhit is highly expressed not only in quiescent but also in active histiocyte. In other words the stable Fhit nuclear expression in histiocytes may be significantly associated with the robust tumour suppressor function, which could explain why the genuine histiocyte-derived tumour is extremely rare. The hypothesis is suggested that the function of Fhit nuclear localization in the monocyte-derived cells be one of signaling molecules for immune response: antigen-presenting and/or cytokine-manufacturing. However, the detailed mechanism between the nuclear localization and biological significance of Fhit in the histiocytes remains for larger studies to resolve.

References

Baffa R., Veronese M.L., Santoro R., Mandes B., Palazzo J.P., Rugge M., Santoro E., Croce C.M. and Huebner K. (1998). Loss of FHIT expression in gastric carcinoma. Cancer Res. 58, 4708-4714.

Barnes L.D., Garrison P.N., Siprashvili Z., Guranowski A., Robinson A.A., Ingram S.W., Croce C.M., Ohta M. and Huebner K. (1996). Fhit, a putative tumor suppressor in humans, is a dinucleoside 5', 5'''-P1, P3-triphosphate hydrolase. Biochemistry 35, 11529-11535.

Bieche I., Latil A., Becette V. and Lidereau R. (1998). Study of FHIT transcripts in normal and malignant breast tissue. Genes Chromosomes Cancer 23, 292-299.

Birrer M.J., Hendricks D., Farley J., Sundborg M.J., Bonome T., Walts M.J. and Geradts J. (1999). Abnormal Fhit expression in malignant and premalignant lesions of the cervix. Cancer Res. 59, 5270-5274

Campiglio M., Pekarsky Y., Menard S., Tagliabue E., Pilotti S. and Croce C.M. (1999). FHIT loss of function in human primary breast cancer correlates with advanced stage of the disease. Cancer Res. 59, 3866-3869

Chaudhuri A.R., Khan I.A., Prasad V., Robinson A.K., Luduena R.F. and Barnes L.D. (1999). The tumor suppressor protein Fhit. A novel interaction with tubulin. J. Biol. Chem. 274, 4378-24382

Chen Y.J., Chen P.H. and Chang J.G. (1998). Aberrant FHIT transcripts in hepatocellular carcinomas.Br. J. Cancer 77, 417-420.

Croce C.M., Sozzi G. and Huebner K. (1999). Role of FHIT in human cancer. J. Clin. Oncol. 17, 1618-1624.

Druck T., Berk L. and Huebner K. (1998). FHITness and cancer. Oncol. Res. 10, 341-345

Fong K.M., Biesterveld E.J., Virmani A., Wistuba I., Sekido Y., Bader S.A., Ahmadian M., Ong S.T., Rassool F.V., Zimmerman P.V., Giaccone G., Gazdar A.F. and Minna J.D. (1997). 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. 57, 2256-2267

Golebiowski F., Kowara R. and Pawelczyk T. (2001). Distribution of Fhit protein in rat tissues and its intracellular localization. Mol. Cell Biochem. 226, 49-55.

Gramantieri L., Chieco P., Di Tomaso M., Masi L., Piscaglia F., Brillanti S., Gaiani S., Valgimigli M., Mazziotti A. and Bolondi L. (1999). Aberrant fragile histidine triad gene transcripts in primary hepatocellular carcinoma and liver cirrhosis. Clin. Cancer Res. 5, 3468-3475.

Greenspan D.L., Connolly D.C., Wu R., Lei R.Y., Vogelstein J.T., Kim Y.T., Mok J.E., Munoz N., Bosch F.X., Shah K. and Cho K.R. (1997). Loss of FHIT expression in cervical carcinoma cell lines and primary tumors. Cancer Res. 57, 4692-4698.

Hadaczek P., Siprashvili Z., Markiewski M., Domagala W, Druck T., McCue P.A., Pekarsky Y., Ohta M., Huebner K. and Lubinski J. (1998). Absence or reduction of Fhit expression in most clear cell renal carcinomas. Cancer Res. 58, 2946-2951

Hao X.P., Willis J.E., Pretlow T.G., Rao J.S., MacLennan G.T., Talbot I.C. and Pretlow T.P. (2000). Loss of fragile histidine triad expression in colorectal carcinomas and premalignant lesions. Cancer Res. 60, 18-21.

Heubner K. and Croce C.M. (2002).FRA3B and other common fragile sites: The weakest links. Nat. Rev. Cancer. 1, 214-221.

Heubner K. and Croce C.M. (2003). Cancer and the FRA3B/FHIT fragile locus: it's a Hit. Br. J. Cancer 88, 1501-1506.

Heubner K., Garrison P.N., Barnes L.D. and Croce C.M. (1998). The role of the FRA3B/FHIT locus in cancer. Ann. Rev. Genet. 32, 7-31.

Heubner K., Druck T., Siprashvili Z., Croce C.M., Kovatich A. and McCue P.A. (1998). The role of deletions at the FRA3B/FHIT locus in carcinogenesis. Recent Results Cancer Res. 154, 200-215

Keck C.L., Zimonjic D.B., Yuan B.Z., Thorgeirsson S.S. and Popescu N.C. (1999). Nonrandom breakpoints of unbalanced chromosome translocations in human hepatocellular carcinoma cell lines. Cancer Genet. Cytogenet. 111, 37-44.

Korsisaari N. and Makela T.P. (2000). Interactions of Cdk7 and Kin28 with Hint/PKCI-1 and Hnt 1 histidine triad proteins. J. Biol. Chem. 274, 34272-34276.

Lee S.H., Kim W.H., Kim H.K., Woo K.M., Nam H.S., Kim H.S., Kim J.G. and Cho M.H. (2001). Altered expression of the fragile histidine triad gene in primary gastric adenocarcinomas. Biochem. Biophys Res. Commun. 284, 850-855.

Menin C., Santacatterina M., Zambon A., Montagna M., Parenti A., Ruol A. and D'Andrea E. (2000). Anomalous transcripts and allelic deletions of the FHIT gene in human esophageal cancer. Cancer Genet Cytogenet. 119, 56-61.

Michael D., Beer D.G., Wilke C.W., Miller D.E. and Glover T.W. (1997). Frequent deletions of FHIT and FRA3B in Barrett's metaplasia and esophageal adenocarcinomas. Oncogene 15, 1653-1659

Negrini M., Monaco C., Vorechovsky I., Ohta M., Druck T., Baffa R., Huebner K. and Croce C.M. (1996). The FHIT gene at 3p14.2 is abnormal in breast carcinomas. Cancer Res. 56, 3173-3179.

Ohta M., Inoue H., Cotticelli M.G., Kastury K., Baffa R., Palazzo J., Siprashvili Z., Mori M., McCue P., Druck T., Croce C.M. and Heubner K. (1996). The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers. Cell 84, 587-597.

Pekrsky Y., Campiglio M., Siprashvili Z., Druck T., Sedkov Y., Tillib S., Draganescu A., Wermuth P., Rothman J.H., Heubner K., Buchberg A.M., Mazo A., Brenner C. and Croce C..M. (1998).Nitrilase and Fhit homologs are encoded as fusion proteins in Drosophila melanogaster and Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 95, 8744-8749.

Sard L., Accornero P., Tornielli S., Delia D., Bunone G., Campiglio M., Colombo M.P., Gramegna M., Croce C.M., Pierotti M.A. and Sozzi G. (1999). The tumor-suppressor gene FHIT is involved in the regulation of apoptosis and in cell cycle control. Proc. Natl. Acad. Sci. USA 96, 8489-8492.

Schlott T., Ahrens K., Ruschenburg I., Reimer S., Hartmann H. and Droese M. (1999). Different gene expression of MDM2, GAGE-1, -2 and FHIT in hepatocellular carcinoma and focal nodular hyperplasia. Br. J. Cancer 80, 73-78

Shi Y., Zou M., Farid N.R. and Paterson M.C. (2000). Association of FHIT (fragile histidine triad), a candidate tumor suppressor gene, with the ubiquitin-conjugating enzyme hUBC9. Biochem. J. 352, 443-448.

Siprashvili Z., Sozzi G., Barnes L.D., McCue P., Robinson A.K., Eryomin V., Sard L., Tagiabue E., Greco A., Fusetti L., Schwartz G., Pierotti M., Carlo M. and Heubner K. (1997). Replacement of Fhit in cancer cells suppresses tumorigenecity. Proc. Natl. Acad. Sci USA 94, 13771-13776.

Sorio C., Baron A., Orlandini S., Zamboni G., Pederzoli P., Huebner K. and Scarpa A. (1999). The FHIT gene is expressed in pancreatic ductular cells and is altered in pancreatic cancers. Cancer Res. 59, 1308-1314 .

Sozzi G., Tornielli S., Tagliabue E., Sard L., Pezzella F., Pastorino U., Minoletti F., Pilotti S., Ratcliffe C., Veronese M.L., Goldstraw P., Huebner K., Croce C.M. and Pierotti M.A. (1997). Absence of Fhit protein in primary lung tumors and cell lines with FHIT gene abnormalities. Cancer Res. 57, 5207-5212.

Tamura G., Sakata K., Nishizuka S., Maesawa C., Suzuki Y., Iwaya T., Terashima M., Saito K. and Satodate R. (1997). Analysis of the fragile histidine triad gene in primary gastric carcinomas and gastric carcinoma cell lines. Genes Chromosomes Cancer. 20, 98-102.

Thiagalingam S., Lisitsyn N.A., Hamaguchi M., Wigler M.H., Willson J.K., Markowitz S.D., Leach F.S., Kinzler K.W. and Vogelstein B. (1996). Evaluation of the FHIT gene in colorectal cancers. Cancer Res. 56, 2936-2939.

Virgilio L., Shuster M., Gollin S.M., Veronese M.L., Ohta M., Huebner K. and Croce C.M. (1996). FHIT gene alterations in head and neck squamous cell carcinomas. Proc. Natl. Acad. Sci. USA 93, 9770-9775

Werner N.S., Siprashvili Z., Fong L.Y., Marquitan G., Schroder J.K., Bardenheuer W., Seeber S., Huebner K., Schutte J. and Opalka B. (2000). Differential susceptibility of renal carcinoma cell lines to tumor suppression by exogenous Fhit expression. Cancer Res. 60, 2780-2785.

Yoshino K., Enomoto T., Nakamura T., Nakashima R., Wada H., Saitoh J., Noda K. and Murata Y. (1998). Aberrant FHIT transcripts in squamous cell carcinoma of the uterine cervix. Int. J. Cancer 76, 176-181.

Yuan B.Z., Keck-Waggoner C., Zimonjic D.B., Thorgeirsson S.S. and Popescu N.C. (2000). Alterations of the FHIT gene in human hepatocellular carcinoma. Cancer Res. 60, 1049-1053.

Zhao P., Song X., Ning Y.Y., Lu Y.L. and Li X.H. (2003). Loss of fragile histidine triad protein in human hepatocellular carcinoma. World J. Gastroenterol., 9: 1216-1219

Zou T.T., Lei J., Shi Y.Q., Yin J., Wang S., Souza R.F., Kong D., Shimada Y., Smolinski K.N., Greenwald B.D., Abraham J.M., Harpaz N. and Meltzer S.J.. (1997). FHIT gene alterations in esophageal cancer and ulcerative colitis (UC). Oncogene 15, 101-105.

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