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Advances in Brief

Loss of *FHIT* Expression in Cervical Carcinoma Cell Lines and Primary Tumors[1]

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Abstract

Allelic deletions involving the short arm of chromosome 3 (3p13–21.1) have been observed frequently in cervical carcinomas. Recently, a candidate tumor suppressor gene, *FHIT* (Fragile Histidine Triad), was cloned and mapped to this chromosomal region (3p14.2). Abnormal *FHIT* transcripts have been identified previously in a variety of tumor cell lines and primary carcinomas, although their significance and the molecular mechanisms underlying their origin remain incompletely defined. In addition, integration of human papillomavirus DNA has been identified at a fragile site (*FRA*3B) within the *FHIT* locus in cervical cancer. These observations motivated us to evaluate *FHIT* mRNA and protein expression in cervical cancer cell lines, primary cervical carcinomas, and normal tissues. Transcripts of the expected size and sequence were the predominant species identified by reverse transcription (RT)-PCR in cultured keratinocytes and all normal tissues evaluated. In contrast, aberrant *FHIT* transcripts were readily demonstrated in 6 of 7 cervical carcinoma cell lines and 17 of 25 (68%) primary cervical carcinomas. Northern blot analyses demonstrated reduced or absent *FHIT* expression in the cervical carcinoma cell lines, particularly those with aberrant RT-PCR products. Immunohistochemical analysis of Fhit expression in cervical tissues revealed strong immunoreactivity in nonneoplastic squamous and glandular cervical epithelium and marked reduction or loss of Fhit protein in 25 of 33 (76%) primary cervical carcinomas. In those cervical cancer cell lines and primary tumors with exclusively aberrant or absent *FHIT* transcripts by RT-PCR, Fhit protein expression was always markedly reduced or absent. The frequent alterations in *FHIT* expression in many cervical carcinomas, but not in normal tissues, suggest that *FHIT* gene alterations may play an important role in cervical tumorigenesis.

Introduction

Worldwide, cervical carcinoma remains the second most common malignancy of women in both incidence and mortality (1). Over the past decade, compelling evidence has been obtained that cervical carcinoma is associated with infection by specific types of HPVs.[4] Molecular studies have begun to elucidate the mechanisms by which HPV infection contributes to cervical tumorigenesis. For example, the E6 and E7 oncoproteins encoded by the high-risk (oncogenic) HPV types have been shown to interact with the tumor suppressor proteins p53 and pRB (2, 3). These protein-protein interactions serve, at least in part, to abrogate cell cycle regulation, particularly the G_1 arrest in response to DNA damage (4–7). Hence, cells infected by high-risk HPVs may be more susceptible to the accumulation of mutations than their uninfected counterparts.

Although HPV infection clearly plays an important role in cervical tumorigenesis, several lines of evidence suggest that HPV infection alone is insufficient for cervical tumor development. For example, most high-risk HPV infections do not result in cervical cancer, and in those individuals who do develop carcinoma, there is usually a relatively long period (years) between initial infection and the identification of invasive disease. Thus, other events such as tumor suppressor gene inactivation and oncogene activation are likely to be critical in the pathogenesis of cervical cancer. Several analyses of cervical carcinoma have shown a high frequency of allelic deletions on the short arm of chromosome 3 (3p13–21.1; Refs. 8–13). Recently, a candidate tumor suppressor gene, *FHIT*, was identified at chromosome 3p14.2 (14). The *FHIT* gene encompasses the t(3;8) breakpoint that segregates with familial renal cell carcinoma, and *FHIT* gene abnormalities have been reported in multiple types of tumor cell lines and primary tumors (14–23). Homozygous deletions affecting *FHIT* coding sequences have been demonstrated in some tumor types, although most studies have focused on the identification of altered *FHIT* transcripts using RT-PCR assays. In addition, integration of HPV DNA has been identified at a fragile region (*FRA*3B) within the *FHIT* gene in cervical cancer (24). To address the hypothesis that *FHIT* may be frequently inactivated in cervical cancers, we characterized *FHIT* mRNA and protein expression in several cervical cancer cell lines, primary cervical carcinomas, and various normal tissues, including normal cervix.

Materials and Methods

Cell Lines and Tissues. All cervical carcinoma cell lines (HT-3, SiHa, C-4 II, CaSki, C-33a, HeLa, and ME-180) were obtained from the American Type Culture Collection and maintained in the recommended media including 10% FBS. Two cell lines (C-33a and HT-3) lack detectable HPV sequences and contain mutant *p53* alleles, whereas the remaining cell lines are HPV positive with wild type p53 (25). Primary keratinocytes were cultured from fresh human foreskins and cervix as described previously (26) and maintained in keratinocyte growth medium (Clonetics, Walkersville, MD). Fresh normal tissues (brain, lung, liver, kidney, colon, thyroid, cervix, ovary, and myometrium) were obtained from surgical specimens and histologically verified as normal by analysis of frozen sections. A total of 35 invasive squamous carcinomas of the cervix [34 primary tumors and 1 metastatic lesion (tumor T17)] were obtained from the Surgical Pathology Department of the Johns Hopkins Hospital, the Department of Obstetrics and Gynecology of the Asan Medical Center, and from a previous study conducted in Spain and Colombia (27, 28). Frozen tissue was available from 25 of the 35 tumors. Regions containing at least 70% tumor cells (assessed by frozen section) were used as sources of RNA. Formalin-fixed, paraffin-embedded material was available from 33 of the 35 tumors.

RNA Extraction and cDNA Synthesis. RNA was extracted from the cervical carcinoma cell lines, selected primary cervical carcinomas, keratinocytes, and normal tissues using TRIzol (Life Technologies, Inc., Grand Island, NY) or RNAgents Total RNA Isolation System (Promega Corp., Madison, WI)

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[4] The abbreviations used are: HPV, human papillomavirus; FHIT, fragile histidine triad; RT-PCR, reverse transcription-PCR; dNTP, deoxynucleotide triphosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

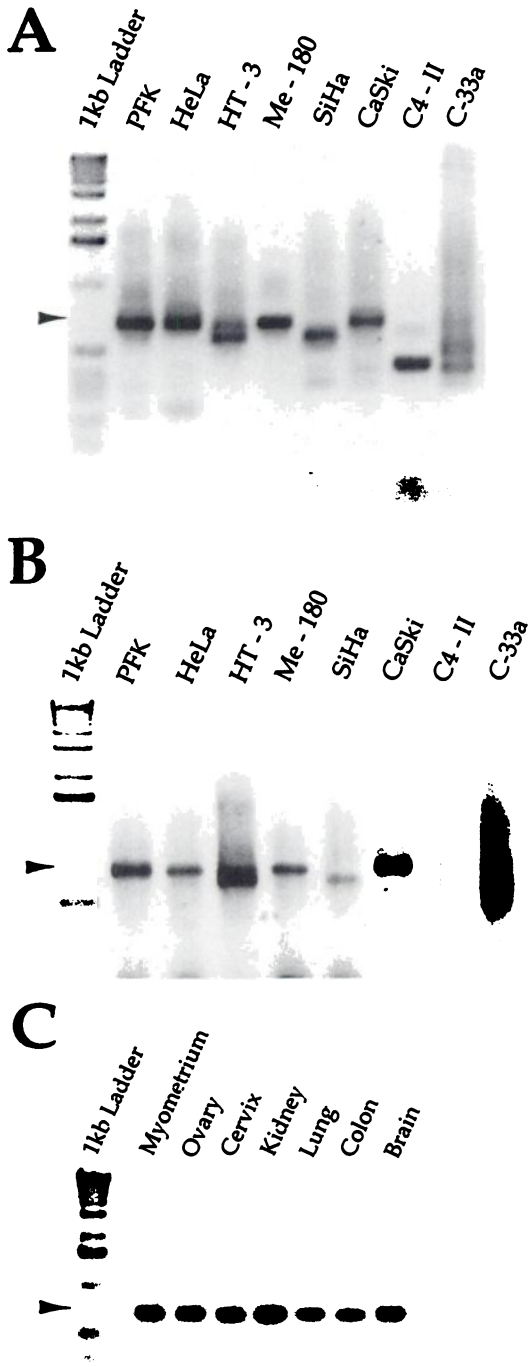

Fig. 1. RT-PCR analysis of *FHIT* expression in cervical carcinoma cell lines and normal tissues. *A*, nested RT-PCR analysis of *FHIT* cDNAs in cervical carcinoma cell lines and primary foreskin keratinocytes (*PFK*). The cell lines evaluated are indicated above the lanes; *left*, 1-kb ladder. *Arrowhead*, size of the expected 707-bp RT-PCR product. *B*, nonnested RT-PCR analysis of cervical carcinoma cell lines and PFK as above. *Arrowhead*, size of the expected 747-bp RT-PCR product. *C*, nested RT-PCR analysis of *FHIT* expression in normal tissues. The specific tissues are indicated above the respective lanes; *left*, 1-kb ladder. *Arrowhead*, size of the expected 707-bp RT-PCR product.

according to the manufacturers' protocols. RNA was solubilized in RNase free water and stored at −80°C. First-strand cDNA was synthesized from 6 μg of DNaseI-treated total RNA. RNA was reverse transcribed in a 30-μl volume of 1× first-strand buffer, 500 μM dNTPs, 0.1 μg/μl random hexamers, 12 units of RNAsin (Promega), 300 units of Superscript II (Life Technologies, Inc.) by incubation at 37°C for 60 min. Each cDNA synthesis reaction was paired with a control reaction without the addition of reverse transcriptase.

RT-PCR and cDNA Sequencing. Amplification of *FHIT* cDNA encompassing the entire open reading frame was performed using both nested (14) and nonnested (29) RT-PCR strategies as described previously. Primers for nested PCR (5U2, 5U1, 3D1, and 3D2) yield expected PCR products of 707 bp, extending from exon 3 into exon 10. Primers for nonnested PCR (MUR5 and RP2) yield expected PCR products of 747 bp, extending from the terminal portion of exon 2 into exon 10. For nested PCR, the first round of cDNA amplification was performed in 25-μl reactions containing 1–2 μl of first-strand cDNA product, 50 μM dNTPs, 1.25 unit of Taq polymerase, and 0.8 μM of *FHIT*-specific primers. Initial denaturation at 95°C for 3 min was followed by 25 cycles of 15 s at 94°C, 30 s at 62°C, 45 s at 72°C, and a final extension of 5 min at 72°C using an Omnigene PCR thermocycler. The amplified products were diluted 20-fold with TE buffer, and 1 μl of diluted product was used in a second round of PCR amplification using nested primers for 30 cycles under the above conditions. The PCR products were evaluated on 2% ethidium bromide-stained agarose gels. Selected PCR products were purified using the Wizard PCR Preps DNA purification kit (Promega), cloned into the pCR2.1 vector, and transformed into One Shot cells using the Original TA cloning Kit (Invitrogen, Omar, UT). Following blue-white selection, plasmid DNA preparations from at least four white transformants per PCR product were each sequenced using the Sequenase version 2.0 sequencing kit (Amersham Corp., Cleveland, OH). Nonnested PCR reactions were performed in 25-μl reaction volumes using 175 ng of each nonnested *FHIT* primer, 300 μM of dNTPs, 2.5 units of Taq polymerase (Life Technologies, Inc.), and 2 μl of cDNA. Initial denaturation for 90 s at 95°C was followed by 35 cycles of 95°C for 30 s, 62°C for 60 s, 70°C for 60 s, and a final extension at 70°C for 5 min. To exclude RNA degradation as an explanation for the absence of *FHIT* expression, RT-PCR using GAPDH-specific primers (product of 508 bp) was performed on all RNA samples. The GAPDH amplifications were performed in 25-μl reaction volumes using 0.4 μM GAPDH-specific primers (sense, 5′-GAGAAGTATGACAACAGCCTC-3′; antisense, 5′-AGTGGTCGTTGAGGGCAATG-3′), 200 μM dNTPs, and 1.5 unit of Taq polymerase. Reactions were initially denatured at 95°C for 2 min and then amplified with 30 cycles of 94°C at 30 s, 60°C for 30 s, 72°C for 2 min, and a final extension at 72°C for 5 min.

Northern Blot Analysis. To analyze *FHIT* mRNA expression in cervical cancer cell lines and primary keratinocytes, 20 μg of total RNA were resolved on 1% agarose-4-morpholinepropanesulfonic acid/formaldehyde gels, transferred to a nylon membrane, and hybridized to a radiolabeled cDNA probe spanning *FHIT* exons 3–9 in Rapid-Hyb buffer (Amersham). The blot was washed at 65°C in 0.3× SSC/0.1% SDS and then autoradiographed. The blot was then stripped of hybridized probe by washing twice in 0.1× SSC/0.5% SDS at 95°C and subsequently rehybridized to a GAPDH cDNA probe to control for potential loading differences between samples. The size of *FHIT* mRNA(s) was assessed by comparison to RNA standards run on the same gel as the RNA samples.

Immunohistochemical Analysis of Fhit Protein Expression. Formalin-fixed, paraffin-embedded cell pellets or tissues were sectioned at 5 μm onto ChemMate-coated slides (Curtin Matheson Scientific, Pittsburgh, PA) and then deparaffinized in two changes of xylene for 5 min each. Sections were hydrated into dH_2O through a series of graded alcohols. Antigen enhancement was performed by steaming the sections at 80°C in sodium citrate buffer (diluted to 1× from 10× heat induced epitope retrieval buffer; Ventana BioTek, Tucson, AZ). Slides were cooled for 5 min and then transferred to a Tech Mate 1000 automated stainer (Ventana-BioTek, Tucson, AZ). Staining was performed at room temperature using the protocol and solutions provided by the manufacturer. Briefly, slides were washed three times in phosphate buffer and then incubated with blocking serum (normal goat serum) for 7 min. Following additional phosphate buffer washes, slides were incubated overnight with primary rabbit polyclonal anti-GST-Fhit antiserum (30) at 1:10,000 dilution (antiserum generously provided by Dr. Kay Huebner, Kimmel Cancer Center, Jefferson Medical College, Philadelphia, PA). Slides were washed again three times and then incubated with secondary antibody (biotinylated goat anti-rabbit IgG) for 30 min. Slides were treated with hydrogen peroxide for 15 min to reduce endogenous peroxidase activity. Avidin-biotin complex was added to the slides for 30 min, and antibody localization was effected by a 20-min incubation with 3,3′-diaminobenzidine. Finally, slides were washed in dH_2O, lightly counterstained with hematoxylin, dehydrated, and then coverslipped using AccuMount (Baxter) mounting medium. For blocking exper-

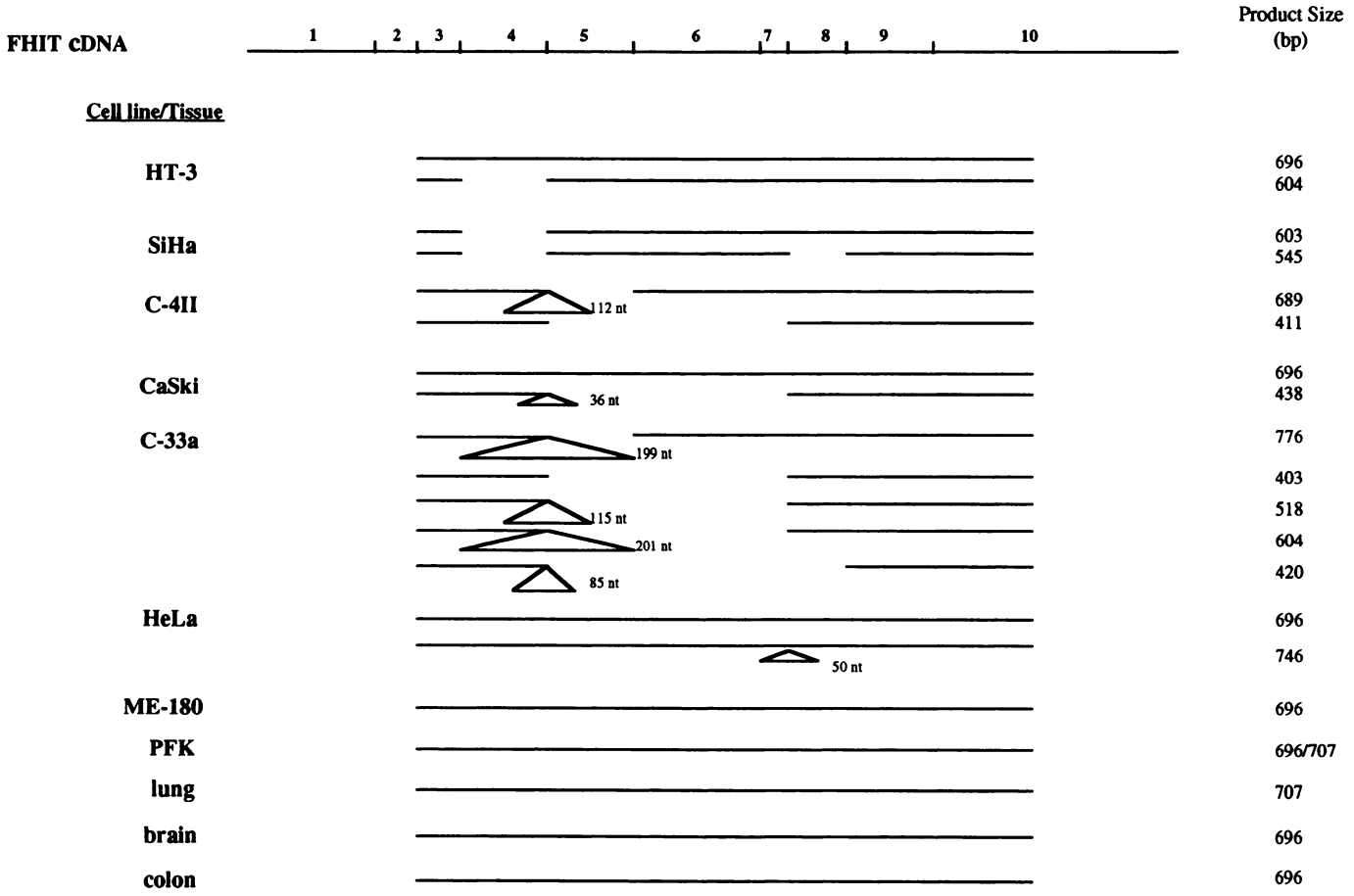

Fig. 2. Schematic diagram of representative transcripts in cervical carcinoma cell lines and selected normal tissues. The structure of the *FHIT* cDNA is shown at the *top*, and the structure and size of representative cDNA clones from the cervical carcinoma cell lines and selected normal tissues are shown *below*. Deleted sequences are represented by *gaps*, and inserted sequences by *triangles*. The size of the full-length RT-PCR product in normal cells was either 696 or 707 bp, depending on the presence or absence of 11 bp in the 3′-untranslated region. This sequence variation, thought to represent alternative splicing of uncertain significance, has been observed previously (17).

iments, 1 μg/ml purified recombinant Fhit protein (generously provided by Dr. Kay Huebner) or irrelevant protein (human milk fat globule protein) was coincubated with the primary antibody. Cell pellets from normal keratinocytes and ME-180 cells (both shown to express only full-length *FHIT* transcripts) and from C-33a cells (shown to express exclusively aberrant *FHIT* transcripts) were used as positive and negative controls, respectively.

Immunostaining was scored on a three-tiered scale for both intensity (absent/weak, 1; moderate, 2; strong, 3) and extent (percentage of positive cells: <10%, 1; 10–50%, 2; >50%, 3). The intensity and extent scores were then multiplied to give a composite score (1–9) for each tumor. Composite scores from 1 to 3 were defined as marked reduction or absence of Fhit protein expression. Slides were scored independently by two pathologists (K. R. C. and D. L. G.).

Results

Aberrant *FHIT* cDNAs Are Frequently Identified in Cervical Carcinoma Cell Lines and Primary Tumors. The predominant *FHIT* transcript in normal tissues encompasses at least 10 exons (30) and is approximately 1.1 kb based on previous Northern blot studies (14). Using RT-PCR assays, *FHIT* cDNAs of altered size and/or sequence were identified in six (HeLa, HT-3, SiHa, C-4II, CaSki, and C-33a) of the seven cervical carcinoma cell lines evaluated (Fig. 1, *A* and *B*). These findings are in agreement with recent studies of cervical carcinoma cell lines (31); however, we found a minor population of aberrant cDNAs in both HeLa and CaSki cells. Both nested (Fig. 1*A*) and nonnested (Fig. 1*B*) PCR strategies yielded similar results, although amplification with the nested strategy was generally more robust. For example, C-4II failed to amplify via the nonnested PCR approach (Fig. 1*B*) but was shown to contain aberrant cDNAs by the nested approach (Fig. 1*A*). In contrast to most carcinoma cell lines, the dominant *FHIT* RT-PCR product detected in primary keratinocytes and all normal tissues examined was of the expected size (Fig. 1*C*). Only cDNAs of the expected size and sequence were identified in the ME-180 cervical carcinoma cell line. Sequence analysis of representative cDNA clones from the cell lines with *FHIT* transcript alterations identified a variety of aberrant species that are summarized schematically in Fig. 2. Exon 5 (containing the *FHIT* translation initiation codon) was often absent, although isolated absence of exon 4 was occasionally seen. Insertions of additional sequence were identified in four of the six cell lines with altered *FHIT* RT-PCR products. These insertions almost always occurred at the boundary between exons 4 and 5, but the novel sequences were not derived from those at this intron-exon boundary. Three cell lines (HeLa, HT-3, and CaSki) showed RT-PCR products of the expected size and sequence in addition to the aberrant ones. One cell line (C-4II) showed a minor population with absence of exon 5 and insertion of nearly the same number of bp at the intron-exon boundary, such that the cDNAs were of the expected size but were, in fact, aberrant.

Seventeen of 25 (68%) primary cervical carcinomas had no detectable *FHIT* transcripts or had transcripts of aberrant size in the RT-PCR assay (Fig. 3). The presence or absence of the RT-PCR products was generally reproducible in independent RT-PCR experiments, but the relative abundance of the altered products was often variable. Transcripts of the expected size identified in primary tumors may reflect normal *FHIT* expression in the neoplastic cells, be derived

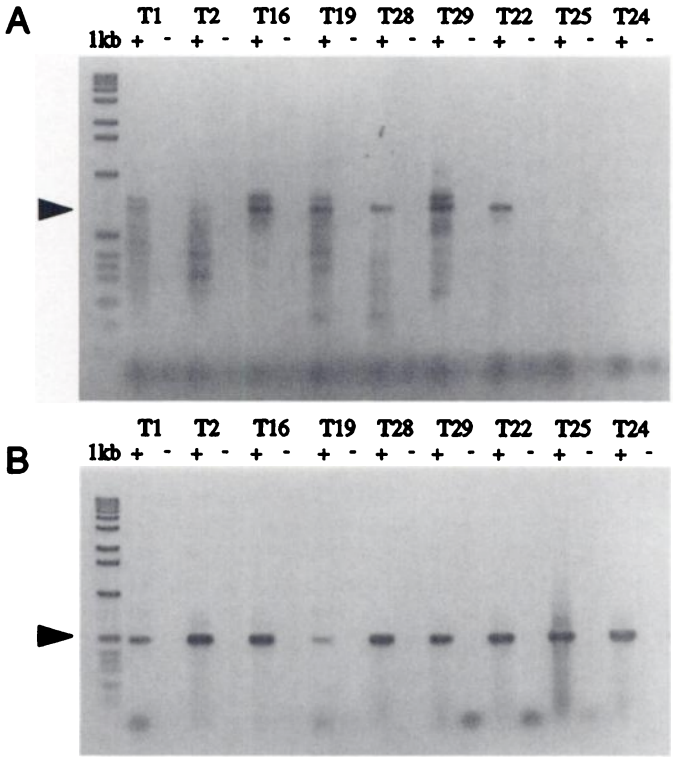

Fig. 3. RT-PCR Analysis of *FHIT* expression in primary cervical carcinomas. *A*, nested RT-PCR analysis of *FHIT* expression in primary tumors. The specific tumors shown are designated above each pair of lanes in which (+) represents reactions performed in the presence of RT and (−) in the absence of RT. *Arrowhead*, size of the expected 707-bp RT-PCR product. *B*, RT-PCR analysis of GAPDH expression in primary tumors. The designation of lanes is identical to that in *A*. *Arrowhead*, size of the expected 508-bp RT-PCR product.

from contaminating normal cells in the tumor specimen, or represent products of the expected size with abnormal sequence.

***FHIT* mRNA Expression Is Markedly Reduced in Most Cervical Carcinoma Cell Lines.** Because of concerns that the RT-PCR assay might not faithfully reflect the relative abundance of *FHIT* transcripts, Northern blot studies were undertaken. Expression of *FHIT* mRNA (≈1.1 kb) was readily detected in primary keratinocytes. In contrast, substantially reduced or absent *FHIT* mRNAs were noted in the cervical carcinoma cell lines (Fig. 4), particularly those cell lines that expressed exclusively aberrant *FHIT* cDNAs in the RT-PCR assay (C-33a and C-4II). A weak, diffuse hybridization signal centered at 4.4 kb was identified equally in all samples in the region of 28S ribosomal sequences. The Northern blot studies of *FHIT* expression in cancer cell lines indicate that the RT-PCR assay, although useful for characterizing altered *FHIT* transcripts, may not be suitable for quantitation of *FHIT* expression levels.

Expression of Fhit Protein Is Markedly Reduced or Absent in Many Primary Cervical Carcinomas. Studies to quantitate *FHIT* mRNA expression in primary carcinomas are hindered by several factors, including lack of sufficient quantities of RNA and concerns regarding RNA quality and contaminating normal cells in the tumor specimen. Therefore, we chose to use immunohistochemical methods to characterize *FHIT* expression *in situ*. The specificity of the anti-Fhit antiserum was initially tested on formalin-fixed, paraffin-embedded cell pellets from cultured normal keratinocytes and the cervical carcinoma cell lines ME-180 and C-33a. As noted above, keratinocytes and ME-180 cells express only *FHIT* transcripts of the expected size, whereas C-33a cells express only aberrant transcripts and have undetectable *FHIT* expression by Northern blot. The anti-Fhit antiserum strongly stained the cytoplasm of normal keratinocytes and ME-180 cells (Fig. 5*A*) but failed to detect Fhit protein in C-33a cells (Fig. 5*B*). Coincubation with excess purified Fhit protein successfully blocked staining in the ME-180 cells (Fig. 5*A*, *inset*). Immunohistochemical analysis of primary cervical tumors and adjacent normal tissues revealed strong cytoplasmic staining of Fhit protein in normal ectocervical squamous epithelium (Fig. 5*C*) and endocervical glandular epithelium (Fig. 5*D*), which was also effectively blocked by coincubation with purified Fhit protein (data not shown). Coincubation with similar concentrations of an irrelevant protein (human milk fat globule protein) had no effect on Fhit immunoreactivity (data not shown). Staining in the squamous epithelium was localized primarily in the differentiating layers, with relative sparing of the basal and parabasal epithelial cells. Although some primary tumors showed Fhit staining similar to that seen in normal cervix (Fig. 5*E*), 25 of 33 (76%) tumors showed marked reduction or absence of Fhit immunoreactivity (Fig. 5*F*). Immunostains of the primary tumors were evaluated independently by two pathologists, and composite scores (≤3 *versus* >3) were concordant in all but 2 of the 33 cases. These two cases were reviewed and reassigned scores based on consensus opinion. The composite score of immunoreactivity correlated quite well with the RT-PCR data in that Fhit protein expression was always reduced or absent (composite score ≤3) in tumors with exclusively aberrant or absent mRNA by RT-PCR (Table 1). Three tumors with substantially reduced Fhit expression contained only transcripts of the expected size by RT-PCR. There are several possible explanations for these observations including: (*a*) the immunohistochemical assay may more accurately reflect Fhit expression than the RT-PCR assay; (*b*) the *FHIT* cDNAs detected were contributed by nonneoplastic contaminating cells; or (*c*) the transcripts of expected size have aberrant sequence and fail to encode Fhit protein. Finally, when both full-length and aberrant transcripts were observed in a tumor, significant immunoreactivity (composite score >3, <9) was sometimes seen, suggesting that some primary tumors, like the carcinoma cell lines, may express both full-length and aberrant *FHIT* mRNAs.

Discussion

Cervical carcinomas, as other tumors, likely result from an accumulation of genetic alterations activating oncogenes and inactivating tumor

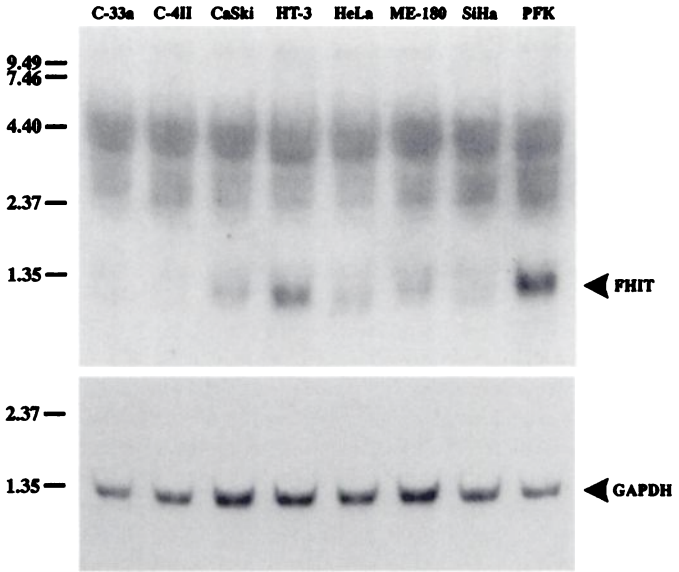

Fig. 4. Northern blot analysis of *FHIT* expression in cervical carcinoma cell lines and primary foreskin keratinocytes. The cell lines evaluated are as indicated. Based on comparison to the migration of RNA standards of known size, the *FHIT* mRNAs were estimated to be approximately 1.1 kb. To control for RNA loading, the blot was rehybridized to a control probe for GAPDH.

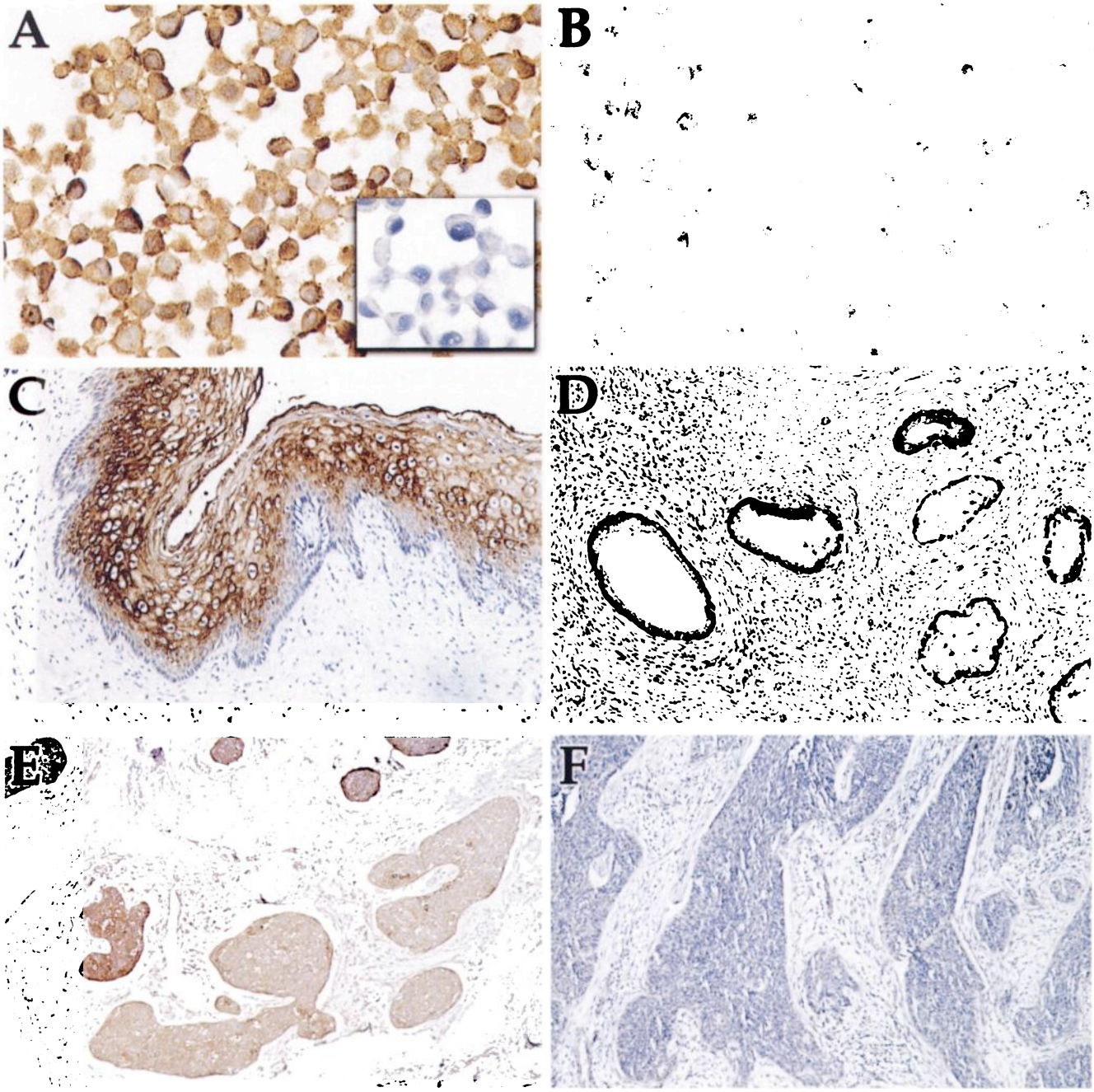

Fig. 5. Representative immunostaining of Fhit protein in selected cell lines, normal cervix, and primary cervical carcinomas. *A*, ME-180 cells showing strong cytoplasmic expression of Fhit protein. Immunoreactivity is blocked by coincubation with excess purified Fhit protein (*inset*). *B*, C-33a cells showing absence of Fhit protein expression. *C*, normal ectocervical squamous epithelium showing immunoreactivity in the differentiating epithelial cells. *D*, benign endocervical glands showing strong immunoreactivity. *E*, representative invasive squamous carcinoma of the cervix with diffuse staining of the tumor cells. *F*, representative immunonegative invasive cervical carcinoma.

suppressor genes and/or genes involved in DNA damage recognition and repair. Although the high-risk HPVs have an important role in cervical cancer, relatively few somatic mutations in cellular genes have been identified. Those alterations that have been identified include amplification of c-*myc* (32), *HER*2-neu (33), and an as-yet-unidentified gene(s) in the distal region of chromosome 3q (34). Allelic losses of chromosome 3p have been seen in a number of analyses of cervical cancer (11–13), and frequent 3p deletions have also been observed in the intraepithelial lesions of the cervix that are believed to be precursors of invasive squamous carcinomas (35). The common regions of allelic loss appear to be 3p13–14.3 and 3p13-p21.1 (9, 35, 36). Despite considerable efforts, the tumor suppressor gene(s) targeted by 3p allelic losses in cervical carcinoma have not been identified conclusively. One of the regions of 3p commonly deleted in cervical carcinomas includes the *FHIT* locus (3p14.2). The *FHIT* gene encompasses a fragile site (*FRA*3B) spanning *FHIT* introns 3–5 (37). Our demonstration of altered *FHIT* transcripts and/or loss of protein expression in most cervical carcinoma cell lines and over 70% of primary cervical tumors supports *FHIT* as a candidate tumor suppressor gene targeted by 3p deletions and/or HPV integration into the *FRA3B* fragile site. Notably, we demonstrated *FHIT* alterations in both HPV-negative and HPV-positive cervical carcinomas.

Several investigators have reported the presence of altered *FHIT* transcripts in primary tumors and cell lines derived from many types of tumors, including those of the lung, breast, head and neck, gastrointestinal tract, and skin (14–18, 20–22, 38). However, only *FHIT* cDNAs were analyzed in these studies, and it has been difficult to

Table 1 *FHIT expression in primary cervical carcinomas*

Tumor no.	Protein			mRNA
	Intensity[a] (1–3)	Extent[b] (1–3)	Composite[c] (1–9)	RT-PCR[d]
T1	3	1	3	ES + AB
T2	2	3	6	ES + AB
T3	2	2	4	ES
T4	2	3	6	ES
T5	3	1	3	
T6	1	3	3	ES + AB
T7	2	3	6	
T8	2	1	2	
T9	3	1	3	ES
T10	3	1	3	
T11	1	1	1	Absent
T12	3	2	6	
T13	1	3	3	
T14	3	1	3	Absent
T15	2	2	4	ES
T16	3	1	3	ES + AB
T17	1	3	3	ES + AB
T18	1	1	1	AB
T19	3	1	3	ES + AB
T20	1	3	3	ES + AB
T21	1	3	3	ES + AB
T22	3	3	9	ES
T23	1	3	3	ES
T24	1	1	1	Absent
T25	1	1	1	Absent
T26	3	2	6	ES
T27	3	1	3	Absent
T28	3	1	3	ES
T29	1	1	1	ES + AB
T30				ES + AB
T31				ES + AB
T32	3	1	3	
T33	3	1	3	
T34	1	1	1	
T35	1	1	1	

[a] Intensity of immunohistochemical staining: 1, absent/weak; 2, moderate; 3, strong.
[b] Extent of immunohistochemical staining (percentage of positive cells): <10%, 1; 10–50%, 2; >50%, 3.
[c] Composite score, intensity × extent.
[d] ES, expected size; AB, aberrant.

assess the origin of full-length transcripts (tumor *versus* contaminating normal cells) in the evaluated specimens. Moreover, the relationship of these altered transcripts to Fhit protein expression has been unclear. In addition to RT-PCR analysis of *FHIT* mRNA expression, we used immunohistochemical methods to assess Fhit protein expression *in situ* in a substantial number of primary cervical carcinomas. We found that expression of Fhit protein is markedly reduced or absent in many primary cervical tumors, supporting the notion that *FHIT* gene inactivation might contribute to clonal outgrowth during cervical tumorigenesis. Finally, the observation that *FHIT* alterations are common in some primary tumor types (*e.g.*, cervical cancer) but are uncommon in others [*e.g.*, esophageal cancer (39) and colorectal cancer (29)] suggest that *FHIT* alterations are not simply a nonspecific marker of genetically unstable malignant cells. This notion is further supported by our preliminary observation that Fhit protein expression is reduced or absent in over 70% of cervical cancers compared to only 20% (2 of 10) of colorectal cancers.[5]

The function of the human Fhit protein is not known, but it bears significant similarity to a yeast diadenosine (Ap_4A) tetraphosphate asymmetrical hydrolase (*Schizosaccharomyces pombe* PAPH1; Ref. 14). Although the status of *FHIT* as a tumor suppressor gene remains controversial, the presence of aberrant *FHIT* transcripts and loss of protein expression in a substantial percentage of cervical cancer cell lines and primary cervical tumors, but not in normal tissues, suggests that *FHIT* gene alterations may play an important role in cervical tumorigenesis. The exact role of *FHIT* in the pathogenesis of cervical cancer awaits further analysis and assessment of the *in vivo* function of the protein in normal, preneoplastic, and neoplastic tissues.

[5] J. T. C. Vogelstein and K. R. Cho, unpublished observations.

Acknowledgments

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