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Alternative Splicing of the *FHIT* Gene in Colorectal Cancers

Kenji Hibi,[1] Masumi Taguchi, Hajime Nakamura, Atsushi Hirai, Yoshihiro Fujikake, Takanori Matsui, Yasushi Kasai, Seiji Akiyama, Katsuki Ito and Hiroshi Takagi

Second Department of Surgery, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466

In the present study, we examined the status of the *FHIT* gene in 112 colorectal cancer and 137 colorectal adenoma specimens. In a total of 5 specimens (4 colorectal cancers and 1 colorectal adenoma), a common smaller product was detected in addition to the normal size product. This smaller product had lost exon 4, the 5′ noncoding region of the *FHIT* gene, owing to alternative splicing. Moreover, all of the 5 tumors with alternative splicing were located lower on the rectum than the anterior peritoneal reflection.

Key words: *FHIT* — Alternative splicing — Colorectal cancer — Colorectal adenoma

There is now good evidence that a series of genetic lesions in both dominant oncogenes and tumor suppressor genes are involved in the pathogenesis of human colorectal cancer. Activation of oncogenes, such as the *ras* and *H19* genes,[1,2] and inactivation of tumor suppressor genes, such as the *APC* and *p53* genes,[3,4] have been identified in colorectal cancer. In addition, several studies have suggested that tumor suppressor loci on chromosome 3p play important roles in human neoplasia[5] and they have been proposed as candidates for designation as tumor suppressor genes.[6,7]

Recently, Ohta *et al.* have identified a novel gene, the *FHIT* gene, at 3p14.2 and reported that 3 of 8 colon cancers exhibited aberrant transcripts of this gene.[8] They also identified this genetic abnormality in many cancers, including those of the esophagus, stomach, lung, and breast, and proposed that the *FHIT* gene may be a tumor suppressor gene.[9,10] Thiagalingam *et al.* re-evaluated the above result and concluded that the nested PCR would result in overrepresentation of shorter transcripts in addition to the normal product.[11] They performed single PCR and reported involvement of the abnormal *FHIT* gene in a maximum of 4 of 31 colorectal cancers (13%). The mechanism of the inactivation of *FHIT* remains unclear.

In the present study, we examined the status of the *FHIT* gene in 112 colorectal cancer and 137 colorectal adenoma specimens. To avoid overestimation of the frequency of the abnormal *FHIT* gene, we performed single PCR, amplifying exons 3–10. In 4 colorectal cancer and 1 colorectal adenoma specimens, a common smaller product was detected in addition to the normal size product, whereas only the normal product was detected in corresponding normal tissues. This smaller product had lost exon 4, the 5′ noncoding region of the *FHIT* gene, owing to alternative splicing.

Moreover, all of the 5 tumors with alternative splicing were located lower on the rectum than the anterior peritoneal reflection.

MATERIALS AND METHODS

Tumor samples Tumor samples together with corresponding normal tissues were collected at the Nagoya University School of Medicine and Nogaki Hospital from 112 colorectal cancer and adenoma patients, who were diagnosed histologically. These samples were obtained during surgery or fiberscopical polypectomy. All tissues were quickly frozen in liquid nitrogen and stored at −80°C until they were analyzed.

RT-PCR analysis RNA was extracted from these tissues, and cDNA was generated from RNA as described previously.[12] The PCR amplification consisted of 30 cycles (95°C for 20 s, 60°C for 30 s, and 72°C for 1 min) after the initial denaturation step (95°C for 1 min). The primers used were: S1 (sense), 5′-TCCGTAGTGCTATCTACAT and AS2 (antisense), 5′-CATGCTGATTCAGTTCCTCTTGG. The PCR amplification was performed as described previously.[13] The PCR products were analyzed by 2% agarose gel electrophoresis.

PCR-SSCP analysis The PCR was performed with oligonucleotide primers in the presence of [^{32}P]dCTP as mentioned above. The PCR products were then electrophoretically separated on a 6% non-denaturing polyacrylamide gel at 5°C.

DNA sequencing Purified PCR products were subcloned in "pCRII." The nucleotide sequences were determined by using a Sequenase Ver. 2.0 sequencing kit.

[1] To whom requests for reprints should be addressed.

The abbreviations used are: PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-polymerase chain reaction; PCR-SSCP, polymerase chain reaction-single strand conformation polymorphism.

RESULTS

We examined the status of the *FHIT* gene in 112 colorectal cancer and 137 colorectal adenoma specimens using RT-PCR analysis. In all of the 112 cancer specimens examined in this study, a normal-sized RT-PCR product with a complete coding region of *FHIT* was observed. In 108 of these specimens, the observed major PCR product was normal, whereas in 4 of the 112 specimens (4%), a common smaller product was also observed in addition to the normal product (Fig. 1A). We further examined the *FHIT* gene in these tumors using PCR-SSCP, as a more sensitive method, and the same result was observed (Fig. 1B). In addition, 1 of the 137 adenoma specimens (1%) also exhibited both a normal and a smaller product. These RT-PCR products were subsequently cloned and we examined the coding sequence of the smaller product. This clone lacked 93 nucleotides compared with the published sequence. Since the endpoints of the missing sequence corresponded precisely to the human *FHIT* gene, we concluded that this clone lacked exon 4, the 5′ noncoding region of the *FHIT* gene (Fig. 2). We further examined the expression level of the *FHIT* gene using northern analysis, but we could not find any difference in expression between the cancers with alternative splicing and corresponding normal tissues (data not shown). In addition, we performed Southern analysis, using the *FHIT* gene probe (exons 3–10), to examine whether the genomic gene showed structural change in these cases with alternative splicing. But we could not detect any genomic change of the *FHIT* gene (data not shown).

DISCUSSION

The *FHIT* gene was found in 1996 by Ohta *et al.*, using the positional cloning method.[8] In their report, they mentioned that aberrant transcripts of the *FHIT* locus frequently existed in colon cancers (3 of 8 colon cancers). Thiagalingam *et al.* pointed out that the nested PCR analysis which Ohta *et al.* performed can result in overrepresentation of shorter transcripts in addition to the normal product.[9] Therefore, Thiagalingam *et al.* performed single PCR and concluded that the abnormality of *FHIT* was confined to at most 4 of 31 colorectal cancers (13%), though the mechanism causing the inactivation of *FHIT* remained unclear. To evaluate these two sets of results, we examined the abnormality of *FHIT* in 112 colorectal cancer and 137 colorectal adenoma specimens using the single PCR method. We found that 4 of 112 colorectal cancer and 1 of 137 colorectal adenoma specimens exhibited a common smaller product in addition to the normal product, and that this smaller product was generated by alternative splicing of the *FHIT* gene, which lacked exon 4, the 5′ noncoding region of the *FHIT* gene. Moreover, 1 colorectal adenoma exhibited alternative splicing, so it was clear that this genetic abnormality could occur in the early stages of tumorigenesis.

The function of the 5′ noncoding sequence is unknown. However, Kozak analyzed the 5′ noncoding se-

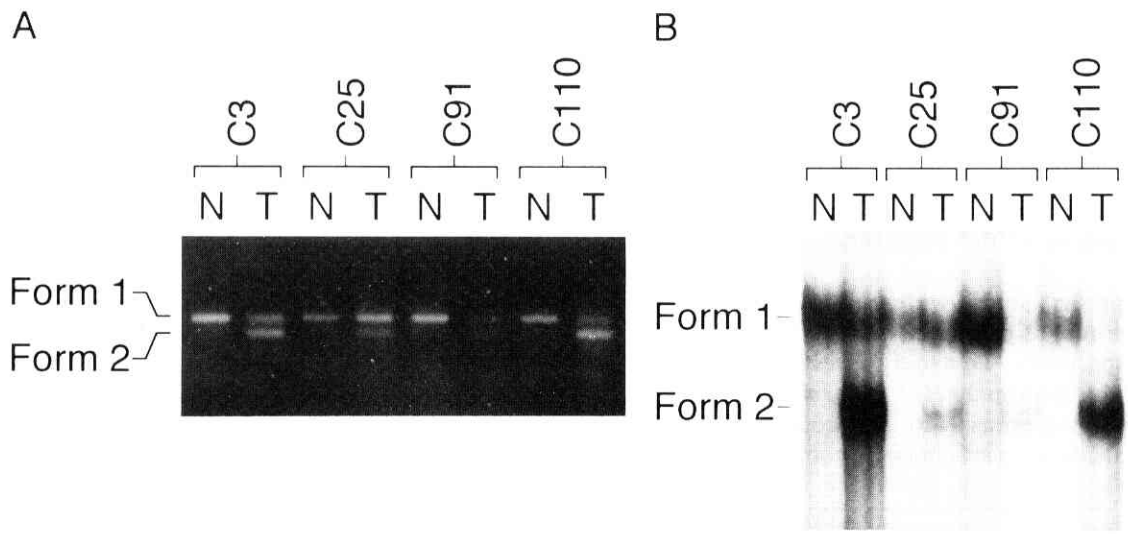
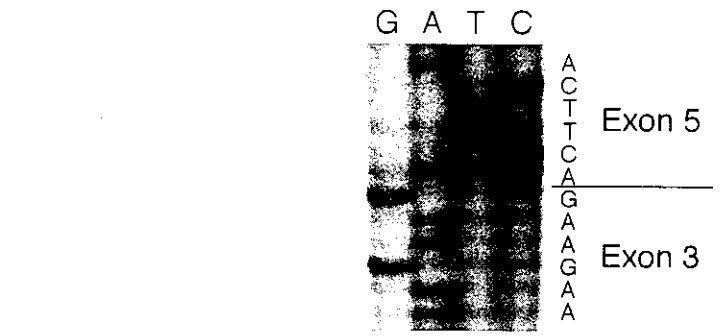


Fig. 1. Expression of the *FHIT* gene in colorectal tumors (T) and paired normal (N) tissues. A, RT-PCR analysis of the *FHIT* gene. Normal tissues exhibit only normal PCR product (form 1, 707 bp), whereas cancer tissues of these 4 cases exhibit a common smaller product (form 2, 614 bp) in addition to the normal product. B, PCR-SSCP analysis of the *FHIT* gene. The same results as in the case of RT-PCR were observed. Case C3 and case C110 showed higher expression of form 2 than form 1.



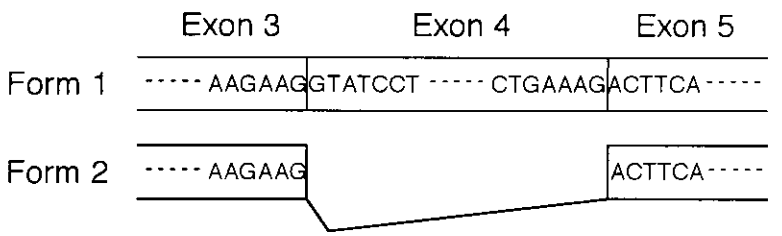


Fig. 2. DNA sequencing of the alternatively spliced *FHIT* mRNA using PCR-amplified cDNA and a schematic diagram of alternative splicing within the 5′ noncoding region.

Table I. Frequency of Alternative Splicing in Colorectal Tumors

Part of colorectum	Form 1 only (%)	Form 1 & 2[a] (%)
Cancer		
Lower on the rectum	18 (82)	4 (18)[b]
Other colorectum	90 (100)	0 (0)[c]
Total	108 (96)	4 (4)
Adenoma		
Lower on the rectum	9 (90)	1 (10)
Other colorectum	127 (100)	0 (0)
Total	136 (99)	1 (1)

a) The significance of the differences was calculated by using Fisher's exact test by comparison with lower on the rectum. *b, c*) $P=0.0012$.

quences of many vertebrate mRNAs and reported in 1991 that the yield of protein initiated from the first AUG codon progressively decreased when the 5′ noncoding sequences of eukaryotic mRNAs were shortened.[14, 15] Therefore, the shortening of the 5′ noncoding sequence caused by alternative splicing may suppress the yield of the FHIT protein. This possibility can be examined in the future by quantitating the FHIT protein.

It is noteworthy that all 5 tumors with alternative splicing were located lower on the rectum than the anterior peritoneal reflection (Table I). Several interpretations of this result are possible. This lower rectal area, referred to as the anal transitional or cloacogenic zone, has extremely variable histology, where columnar, transitional, or squamous epithelium may be found.[16] This difference from other colorectal regions may be related to the formation of alternative splicing. In addition, this lower rectal area is always exposed to the physical stimulation of defecation, and this may be related to the formation of alternative splicing. As the frequency of alternative splicing is 18% in lower cancers on the rectum, alternative splicing could have an important role in these cancers (Table I).

In this report, we have shown that an alternative splicing form of the *FHIT* gene, lacking exon 4, exists in colorectal cancer and adenoma, and all tumors with the alternative splicing were located lower on the rectum. Additional studies, such as measurement of the FHIT protein, may confirm the inactivation process of *FHIT* in colorectal cancer.

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