Advances in Brief

**FHIT Mutations in Human Primary Gastric Cancer**

Akihiko Gemma, Koichi Hagiwara, Yang Ke, Louise M. Burke, Mohammed A. Khan, Makoto Nagashima, William P. Bennett, and Curtis C. Harris

Laboratory of Human Carcinogenesis, Division of Basic Sciences, National Cancer Institute, NIH, Bethesda, Maryland 20892-4255 (A. G., K. H., L. M. B., M. A. K., M. N., W. P. B., C. C. H.); and School of Oncology, Beijing Medical University, Beijing 100034, China (Y. K.)

**Abstract**

Allelic deletion of multiple regions on the short arm of chromosome 3 (3p) implies the presence of multiple important tumor suppressor genes in human carcinogenesis. The FHIT gene, identified recently in chromosome 3p14.2, shows frequent allelic deletion and aberrant transcripts in gastrointestinal tumors. After determining the intron sequences flanking each of the coding exons of the FHIT gene and designing intron primers to facilitate mutation analysis of genomic DNA samples, we analyzed the complete coding sequences in matched cancer and normal tissues from 40 cases with primary gastric cancer using intron primers, PCR-single-strand conformation polymorphism analysis, and direct sequencing. A somatic missense mutation in exon 6, codon 61, (methionine) → (asparagine) was found in a signet ring cell adenocarcinoma. We also evaluated allelic deletion in these tumors by PCR-based microsatellite analysis; allelic deletion occurred in 42.1% (16 of 38) of evaluable cases. This is the first report of a somatic missense mutation of the FHIT gene in a primary tumor. Presence of a point mutation and frequent allelic deletions are consistent with the hypothesis that FHIT gene alterations are involved in the development of primary gastric cancers.

**Introduction**

Allelic losses at multiple sites of chromosome 3p in many types of human cancers (1–7) are consistent with the hypothesis that tumor suppressor genes are located on chromosome 3p. Several loci of homozygous deletion have been reported at chromosome 3p21–25 (8–10). To date, only one tumor suppressor, the VHL (von Hippel-Lindau disease) gene at chromosome 3p25, has been identified, and it is associated with renal cancer and hemangioblastoma (11–13).

A reciprocal chromosomal translocation, t(3;8)(p14.2; q24), which segregated with familial renal cell carcinoma, has been reported (14, 15). Chromosome 3p14.2 also is an aphidicolin-inducible fragile site, FRA3B (16). Recently, the FHIT gene was identified at chromosome 3p14.2, and aberrant transcripts in gastrointestinal tumors were reported (17). The FHIT protein is 69% similar to diadenosine 5',P',P''-tetraphosphate asymmetrical hydrolase, which led to speculation that it cleaves the diadenosine 5',5''-P',P''-tetraphosphate substrate that may stimulate DNA polymerase activity (18). In addition, lung cancers (18), breast cancers (19), and Merkel cell carci

**Materials and Methods**

**Determination of Intronic Sequences.** The intronic sequences of the FHIT gene were determined by comparing the sequence of the FHIT cDNA (17) and the sequences obtained from human mega-YAC2 clones (yhCEPH768D2 and yhCEPH666C1) spanning the FHIT coding region as described previously (22). yhCEPH768D2 and yhCEPH666D2 were identified by screening a human mega-YAC library from Centre d'Etudes du Polymorphisme Humain (thus, CEPH) using PCR primer pairs for exons 5 and 9 of FHIT. The primers were as follows: exon 5, 5'-GCCAAGATCTCATCAAGCC-3' (sense) and 5'-GTACACAGGTTCCTATCAC-3' (antisense); and exon 9, 5'-TGACAGAGGACCTTTCTCG-3' (sense) and 5'-TGTGCACTGAAGATGCC-3' (antisense). We sequenced DNA using the "long distance sequencer" method (23). In brief, DNAs from YAC clones were digested with multiple restriction enzymes and then ligated to vectorette bubbles. The DNAs were amplified with an exon-specific and a vectorette-specific primer containing the M13 sequence. 5'-TGTTAAAACGACGGCCC-3', using the Gene Amp XL PCR kit (Perkin-Elmer/Roche, Branchburg, NJ). The two or three amplified fragments were sequenced directly from a vectorette-specific primer using a fluorescent automated sequencer (model 370A; Applied Biosystems, Inc., Foster City, CA). The PCR conditions were as follows: 40 s at 94°C, 30 s at 60°C, and 90 s at 68°C for 40 cycles, followed by 8 min at 68°C. The PCR reaction mix consisted of 1× XL buffer from the Gene Amp XL PCR kit, 200 μM deoxynucleotide triphosphate, 1100 μM Mg(OAc)2, 0.5 units of rTth DNA Polymerase XL, 0.3 μM of primers, and 25 ng of YAC DNA.

**Tissue Samples.** Matched primary gastric cancers and normal tissues were obtained from 40 Chinese patients resented at the School of Oncology, Beijing Medical University. The histological types included 4 well-differentiated, 8 moderately differentiated, and 7 poorly differentiated, and 27 poorly differentiated adenocarcinomas and 1 signet ring cell adenocarcinoma. Thirty-five tumors were of advanced stage with regional or distant metastases. Genomic DNA samples were isolated by proteinase K treatment and phenol chloroform extraction using standard protocols.

**PCR-SSCP Analysis, Sequencing, and Allelic Deletion Analysis.** PCR-SSCP and DNA sequence analyses were performed as described previously (24). PCR primer pairs for each exon were designed using the flanking intron sequences determined in this study. The PCR conditions were the same as described above. The PCR reaction mix consisted of 1× XL buffer, 200 μM deoxynucleotide triphosphate, 1100 μM Mg(OAc)2, 0.5 units rTth DNA Polymerase XL, 0.3 μM primers, 10 μCi of [α-32P]dCTP, and 25 ng of genomic DNA. The PCR products were then denatured, cooled on ice, loaded on 6% polyacrylamide gels with and without 5% (vol/vol) glycerol, electrophoresed, dried, and exposed to X-ray films at —80°C overnight. The aberrant bands were excised and amplified by PCR using sequencing primers with the M13

Received 10/22/96; accepted 2/28/97.

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1To whom requests for reprints should be addressed, at Laboratory of Human Carcinogenesis, National Cancer Institute, NIH, Building 37, Room 2C05, 37 Convent Drive, MSC 4255, Bethesda, MD 20892-4255. Phone: (301) 496-2048; Fax: (301) 496-0497; E-mail: Curtis.Harris@nih.gov.

2The abbreviations used are: YAC, yeast artificial chromosome; SSCP, single-strand conformation polymorphism.
Table 1: Flanking intron sequences and primers for FHIT gene exons

**EXON 5**
cattgtacctaaaacacccatggtcttctctctctcagactgcatgctttgttaaggctgtatgtatt
tatatagttggaaatctcgcttttcttttttaggctatgtttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
markers on chromosome 3p: D3S1300 is located within the FHIT gene; D3S1312 is centromeric; and D3S1234, D3S1295, and D3S1313 are telomeric. Allelic deletions occurred in 7 of 24 (29.2%) informative cases for D3S1324, 5 of 18 (27.8%) for D3S1295, 8 of 29 (27.6%) for D3S1300, 4 of 22 (18.2%) for D3S1312, and 4 of 10 (40.0%) informative cases for D3S1313 (Fig. 2). Fig. 3 shows the patterns of deletion for all 16 cases. It is notable that 8 of 13 informative cases have a deletion at D3S1300 that occurs within intron 5 of the FHIT gene. It is notable that the remaining eight cases have deletions at adjacent microsatellite or are noninformative. Therefore, these data support the hypothesis that FHIT is the target of deletion in these primary gastric cancers. The overall allelic deletion rate of 42.1% (16/38) in this study correlates with the allelic deletion rate of 42.1% (16/38) in this study correlates with the 42.1% in other studies.

There are several possible interpretations of our data. First, FHIT is a target of mutational inactivation in 40–50% of gastric tumors; our data suggest that usually there is deletion of both alleles, and occasionally there is deletion of one allele with mutational inactivation of the second. The second possibility is that FHIT is mostly altered by nonmutational mechanisms causing down-regulation of protein expression. Lastly, FHIT alterations are not involved in the molecular pathogenesis of gastric cancer, and it is altered only because it is near a tumor suppressor gene. Future studies need to focus on the functional analysis of the FHIT gene as a tumor suppressor, including comparing the wild-type to the missense mutant observed in tumors.

Acknowledgments

We appreciate the editorial assistance of Dorothea Dudek.

References

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