

# Translocation Breakpoints in *FHIT* and *FRA3B* in Both Homologs of Chromosome 3 in an Esophageal Adenocarcinoma

Jianming M. Fang,<sup>1,2</sup> Martin F. Arlt,<sup>1,2</sup> Ann C. Burgess,<sup>1</sup> Susan L. Dagenais,<sup>1,2</sup> David G. Beer,<sup>3</sup> and Thomas W. Glover<sup>1,2\*</sup>

<sup>1</sup>Department of Pediatrics, University of Michigan, Ann Arbor, Michigan

<sup>2</sup>Department of Human Genetics, University of Michigan, Ann Arbor, Michigan

<sup>3</sup>Department of Surgery, University of Michigan, Ann Arbor, Michigan

Common fragile sites have been proposed to play a mechanistic role in chromosome translocations and other rearrangements in cancer cells in vivo based on their behavior in vitro and their co-localization with cancer translocation breakpoints. This hypothesis has been the subject of controversy, because associations have been made at the chromosomal level and because of the large number of both fragile sites and cancer chromosome breakpoints. Tests of this hypothesis at the molecular level are now possible with the cloning of common fragile site loci and the use of fragile site clones in the analysis of rearranged chromosomes. *FRA3B*, the most frequently seen common fragile site, lies within the large *FHIT* gene. It is now well established that this region is the site of frequent, large intragenic deletions and aberrant transcripts in a number of tumors and tumor cell lines. In contrast, only one tumor-associated translocation involving the *FHIT* gene has been reported. We have found translocations in both homologs of chromosome 3 in an early-passage esophageal adenocarcinoma cell line. This cell line showed no normal *FHIT* transcripts by reverse transcription polymerase chain reaction. Subsequent chromosome analysis showed translocations of the short arms of both homologs of chromosome 3: t(3;16) and t(3;4). The breakpoints of both translocations were shown by fluorescence in situ hybridization and polymerase chain reaction to be in the *FHIT* gene, at or near the center of the fragile site region. Using rapid amplification of cDNA ends with *FHIT* primers, a noncoding chimeric transcript resulting from t(3;16) was identified. These data provide direct support for the hypothesis that *FRA3B*, and likely other common fragile sites, may be "hot spots" for translocations in certain cancers, as they are for deletions, and that such translocations have the potential to form abnormal chimeric transcripts. In addition, the results suggest selection for loss of a functional *FHIT* gene by the translocation events. © 2001 Wiley-Liss, Inc.

## INTRODUCTION

It is well established that common fragile sites are loci that are especially sensitive to forming gaps or occasionally breaks on metaphase chromosomes when cultured with aphidicolin or under conditions of folate stress (Glover, 1998). Consistent with a possible role in chromosome rearrangements in vivo, they also have been shown in cells cultured in vitro to be "hot spots" for translocations and deletions (Glover and Stein, 1988; Wang et al., 1997), sister-chromatid exchanges (SCEs) (Glover and Stein, 1987), DNA integration (Rassool et al., 1991), and DNA breaks associated with gene amplification in CHO cells (Coquelle et al., 1997). The association between the location of common fragile sites and sites of chromosome translocation or other rearrangements in cancer and leukemia was noted by many investigators when common fragile sites were first characterized (Glover et al., 1984; Hecht and Glover, 1984; Le Beau and Rowley, 1984; Yunis and Soreng, 1984). This coincident location of fragile sites and recurrent cancer-related breakpoints led to the hypothesis that there is

a mechanistic relationship between the two. This hypothesis was advanced most noticeably by Yunis and Soreng (1984), who promoted it by noting a large number of such associations in numerous leukemias, lymphomas, and solid tumors. This hypothesis, however, has been the subject of controversy, owing to the sheer number of both breakpoints and fragile sites and the fact that the location of fragile sites was based on conventional cytogenetic assignments and not on molecular maps.

The cloning of common fragile site regions has allowed more sensitive molecular analysis of their behavior in cancer cells. The *FRA3B* fragile site lies within the *FHIT* gene, which has been shown to be mutated by large intragenic deletions and to produce aberrant transcripts in a variety of tumor

Supported by: National Cancer Institute; Grant number: CA43222.

\*Correspondence to: Dr. Thomas W. Glover, Department of Human Genetics, Box 0618, 4909 Buhl, University of Michigan, Ann Arbor, MI 48109–0618. E-mail: glover@umich.edu

Received 7 September 2000; Accepted 11 September 2000

cells (Sozzi et al., 1998). The deletions suggest that instability at the fragile site is mechanistically responsible for the deletions and also that there is selection for loss of functional *FHIT* alleles in these tumor cells. Recent investigations of other common fragile sites show a similar pattern of deletion in cancer cells, in agreement with the hypothesis that this is a general feature of common fragile sites (Huang et al., 1999; Mangelsdorf et al., 2000; Paige et al., 2000). For the most part, the rearrangements studied and reported to date are large deletions of hundreds of kilobases.

Few attempts have been made to identify translocations or other gross chromosomal rearrangements at fragile sites, which, if found, would support original suggestions concerning fragile sites and cancer-related chromosome rearrangements. Only two translocations have been reported previously, with breakpoints shown to be in the *FHIT*/FRA3B region. One of these, t(3;8)(p14.2;q24), was a constitutional translocation associated with hereditary renal cell cancer in one family (Cohen et al., 1979), which was found to result in a chimeric transcript between *FHIT* and the *TRC8* gene, a putative *patched* family member (Gemmill et al., 1998). The second was in a pleomorphic adenoma of the thyroid gland, which resulted in the fusion of *FHIT* and the *HMGIC* gene on chromosome 12 (Geurts et al., 1997). Recently, the FRA16D region has been shown to contain a gene termed *FOR*, with homology to the oxidoreductase gene family, within which three of five previously mapped multiple myeloma translocation breakpoints are located (Mangelsdorf et al., 2000).

We report here the finding of two different translocation breakpoints within FRA3B and the *FHIT* gene in both homologs of chromosome 3 in an early-passage esophageal adenocarcinoma cell line. Characterization of the two translocations showed one to result in a nonfunctional chimeric transcript with sequences at 16p13.3. The second is a translocation to a region on distal 4p. These results provide further evidence that fragile sites participate in translocations in tumor cells. Together with earlier findings of translocations within fragile site regions in cancers, they suggest that such events may not be infrequent, and they provide a mechanism for gene loss or formation of novel transcripts.

## MATERIALS AND METHODS

### Cell Lines

The BIC-1 adenocarcinoma cell line was established from minced tumor tissue obtained from a

male patient who had undergone surgical resection for a stage T3, N1, MO Barrett adenocarcinoma and who had received no other preoperative treatments, such as radiation or chemotherapy. The cell line was grown in Dulbecco modified Eagle medium with 15% fetal bovine serum and analyzed at passages 8–10.

### Cytogenetic Analysis and Fluorescence In Situ Hybridization

Chromosome preparations and G-banding were done by standard methods, and fluorescence in situ hybridization (FISH) was done as previously described (Dagenais et al., 1999). For FISH probes, yeast artificial chromosome (YAC) y850A6 was mapped to FRA3B as previously described (Wilke et al., 1994), and YAC y750F1 was obtained from Research Genetics (Huntsville, AL). To obtain a FISH probe specific for *FHIT* exon 10, a human DNA lambda library was screened with *FHIT* exon 10 cDNA as a probe by conventional filter hybridization. A 15-kb genomic fragment containing part of intron 9, exon 10, and 3'-flanking sequence was isolated and used as a probe for FISH.

FISH probes for *FHIT* intron 5 were obtained by screening of the CITB human bacterial artificial chromosome (BAC) library (Research Genetics) by polymerase chain reaction (PCR) methods using primers within intron 5. Clone 254E1 was identified by screening with PCR primers (5'-GAAGATGAG-CAGCGTATGTTGC-3' and 5'-TGTCCTAGGT-GCTGAGGACAC-3'), which are approximately 50 kb distal to exon 5. Clone 385N7 was identified using PCR primers (5'-GGATCTCTCATCCAACCT-TCC-3' and 5'-GATCCCTGGGGTGTAACCTA-TG-3'), which are approximately 150 kb distal to exon 5. Thus, BAC clone 254E1 contains proximal intron 5 sequences, whereas clone 385N7 contains sequences from the central region of intron 5.

Whole-chromosome paint probes to chromosomes 3 (Imagenetics, Framingham, MA), 4 (Oncor, Gaithersburg, MD), and 16 (Oncor) were hybridized separately and in combination according to the manufacturer's instructions. The chromosome 3 probe was directly labeled with Spectrum Orange, and the chromosome 4 and 16 probes were detected with fluorescein isothiocyanate-anti-digoxigenin. Chromosomes were counterstained with 4'6-diamidino-2-phenylindole.

### Genomic PCR

Genomic DNA was extracted from the BIC-1 cell line using the QIAamp Tissue Kit (Qiagen, Valencia, CA). PCR was done using markers from *FHIT* intron

4 (D3S1481), *FHIT* intron 5 (D3S4103), and *FHIT* exon 5 (forward primer: 5'-ATGTCGTTTCAGATT-TGGCC-3'; reverse primer: 5'-CTGGTACCACAG-GTTTCCTA-3').

#### Reverse Transcription-PCR and Rapid Amplification of cDNA Ends

Total RNA was isolated from the BIC-1 cell line using Trizol (Gibco BRL, Gaithersburg, MD) according to the manufacturer's instructions. Reverse transcription-PCR (RT-PCR) was done using primers from *FHIT* exons 1 and 10. Three pairs of primers were used for PCR, with the first pair containing the exon 1 forward primer (5'-CTTTT-TGCCCTCTGTTCCCG-3') and exon 1 reverse primer (5'-GGATGTTGACAGCTGGGAATG-3'). The second pair contained the exon 10 forward primer (5'-CCTGAATTCAGCAAAGAGC-3') and the exon 10 reverse primer (5'-GCAGCAGAG-GAAGGAAGTTTA-3'). The third pair contained the exon 1 forward primer (5'-CTTTT-TGCCCT-CTGTTCCCG-3') and the exon 10 reverse primer (5'-TCACTGGTTGAAGAATACAGG-3').

We used the 3' RACE system for the rapid amplification of cDNA ends (Gibco BRL). Briefly, first-strand cDNA was reverse-transcribed from 2 µg of total RNA, using the oligo dT-adapter primer supplied by the manufacturer. For 3' RACE, amplification of the target cDNA was done using a forward primer to exon 1 of *FHIT* (5'-CTTTT-TGCCCTCTGTTCCCG-3') and a reverse primer complementary to the adapter. 5' RACE was similarly done using a 5' RACE system (Gibco BRL). First-strand cDNA was reverse-transcribed using a primer specific for *FHIT* exon 10 (5'-TGCCCTGT-CTGAGCCGTTTtaggtctagg-3'). A poly-C tail then was added to the purified first-strand cDNA in a TdT tailing reaction. Amplification of the target cDNA was performed with the 5' RACE anchor primer containing poly-G (supplied by the manufacturer) and a nested primer from *FHIT* exon 10 (5'-TCACTGGRRGAAGAATACAGG-3'). All RACE products were cloned into plasmid vector pCR2.1 (Invitrogen, Carlsbad, CA).

#### Sequencing

All sequencing was done by the Sequencing Core Laboratory at the University of Michigan using ABI 377 automated sequencers.

### RESULTS

G-banded chromosome analysis of the early-passage BIC-1 esophageal adenocarcinoma cell line showed a hyperdiploid karyotype with a number of

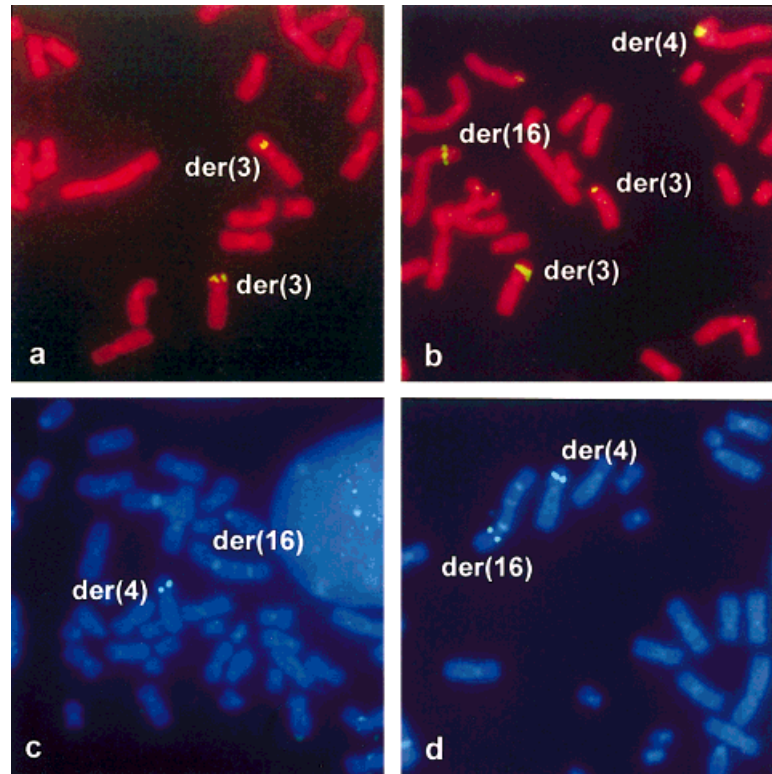
chromosomal rearrangements. The karyotype, as determined by G-banding and FISH with FRA3B probes, is: 48-50,XX,-Y,+X,t(3;4)(p14.2;p16), der(3)t(3;16)(p14.2;p13.3),-5,+add(5)(q13)x2, del(7)(p12), der(8)t(8;13)(p21;q12),der(10)t(10;14)(q22;q11)ins(10;?)(q22;?)x2,-14,-14,der(15)t(14;15)(q11;q13),der(16)t(3;16)(p14.2;p13.3)idic(16)(q24)add(16)(p11),add(18)(p11),+3mar[cp6].

Both homologs of chromosome 3 were rearranged, with translocation breakpoints at or near 3p14.2 and reciprocal breakpoints at 4p16 and at 16p13 of an isodicentric chromosome 16. The der(16) had undergone an additional rearrangement, with the presence of additional material of unknown origin translocated to 16p11 of the other arm of the isodicentric chromosome. RT-PCR amplification of the entire *FHIT* transcript using primers from exons 1 and 10 resulted in no product, whereas RT-PCR using primers within exons 1 and 10 separately gave fragments of the expected sizes (data not shown). FISH with whole-chromosome paint probes (not shown) indicated a reciprocal translocation between 3p and der(16) and translocation of chromosome 3 material to 4p. Chromosome 4 or 16 signal was not detected on the other der(3) chromosome with the paint probes used.

These findings led us to look for translocations within the *FHIT*/FRA3B region using region-specific YAC clones in FISH experiments. YAC y850A6 extends from a region proximal (centromeric) to the *FHIT* locus to the proximal (5') end of intron 5. YAC y750F1 extends from *FHIT* intron 4 to a region distal to the *FHIT* locus (Ohta et al., 1996). Both YACs span FRA3B gaps on normal metaphases. Using these YACs as probes on BIC-1 metaphase cells, y750F1 was found to span the translocation breakpoints of both chromosome 3 homologs and showed the reciprocal translocation breakpoints to be at 4p16 and 16p13 (Fig. 1). Because y750F1 extends distally past *FHIT*, a *FHIT* exon 10 FISH probe also was used to confirm that the breakpoints were indeed within the *FHIT* gene (not shown). Exon 10 was translocated to the der(4) and der(16) chromosomes, showing that the breakpoints were between intron 4 and exon 10 of the *FHIT* locus (not shown).

To define further the location of the breakpoints within the *FHIT*/FRA3B region on chromosome 3, we performed FISH with BAC clone probes from within the large (~500 kb) intron 5 of *FHIT* and genomic PCR with *FHIT* primers. The results showed that BAC 254E1, containing proximal intron 5 sequences, hybridized only to the der(4) chromosome, whereas BAC 385N7 from the mid-

Figure 1. Fluorescence in situ hybridization results show translocation breakpoints involving both homologs of chromosome 3. **a:** YAC y850A6 hybridizes to both der(3) chromosomes. **b:** YAC y750F1 hybridizes to both der(3) chromosomes and the der(4) and der(16) translocation partners, showing that it spans both translocation breakpoints. **c:** BAC 254E1 hybridizes only to the der(4) chromosome, showing that it lies distal to one chromosome 3 breakpoint and indicating a proximal intron 5 deletion in the homolog. **d:** BAC 358N7 hybridizes to both the der(4) and der(16) chromosomes, showing that it lies distal to the breakpoint on both chromosomes 3.



dle of intron 5 hybridized to both the der(4) and der(16) chromosomes (Fig. 1). Genomic PCR was done using primers from *FHIT* intron 4 (D3S1481), *FHIT* intron 5 (D3S4103), and *FHIT* exon 5. PCR amplification of D3S1481 and D3S4103 gave fragments of the expected sizes. PCR amplification using *FHIT* exon 5 primers, however, resulted in no fragments, suggesting that exon 5 is homozygously deleted in BIC-1. This deletion was confirmed by Southern blot hybridization of BIC-1 DNA with an exon 5 probe (data not shown).

Taken together, the FISH and genomic PCR data show that both homologs of chromosome 3 are broken in the region from intron 4 to proximal to intron 5, and both translocations are accompanied by different deletions of the region around exon 5 (Fig. 2). The t(3;4) breakpoint lies proximal to BAC 254E1 and is accompanied by a deletion that encompasses *FHIT* exon 5. The t(3;16) breakpoint is proximal to BAC 385N7 and is accompanied by a larger deletion that encompasses exon 5 and the region of intron 5 contained in BAC 254E1. The proximal boundary of both translocations is *FHIT* intron 4, or the proximal end of y750F1, since this YAC spans both translocation breakpoints.

5' RACE from *FHIT* exon 10 showed only aberrant *FHIT* transcripts and no chimeric transcripts. The aberrant transcript contained 93 nucleotides of

*FHIT* intron 8 with *FHIT* exons 9 and 10. The aberrant transcript may arise from a promoter-like region in intron 8. Using 3' RACE, no transcripts containing chromosome 4 sequences were noted, but novel chimeric transcripts resulting from the t(3;16) were identified. These contained exons 1, 2, and 4 of the *FHIT* gene and additional sequences identical to GenBank sequences from BAC clone 185J20, which map to 16p13.3. The chromosome 16 sequences were spliced from three putative exons (TE1, TE2, and TE3), with one chimeric transcript clone containing TE1 and TE3, a second clone containing TE2 and TE3, and a third containing only TE3 (Fig. 3). These three chimeric transcripts appear to result from alternative splicing.

The sequence of the longest 3' RACE product containing *FHIT* exons 1, 2, and 4 and cryptic exons TE1 and TE3 from 16p13.3 is shown in Figure 4. TE1, TE2, and TE3 contain stop codons in all three reading frames, with the longest open reading frame containing 52 amino acids. Northern blot analysis with TE1, TE2, and TE3 as probes did not detect a positive signal on a human multiple tissue Northern blot (Clontech, Palo Alto, CA) containing brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, lung, and peripheral blood leukocytes. Thus, TE1-TE3 does not appear to represent a functional gene but



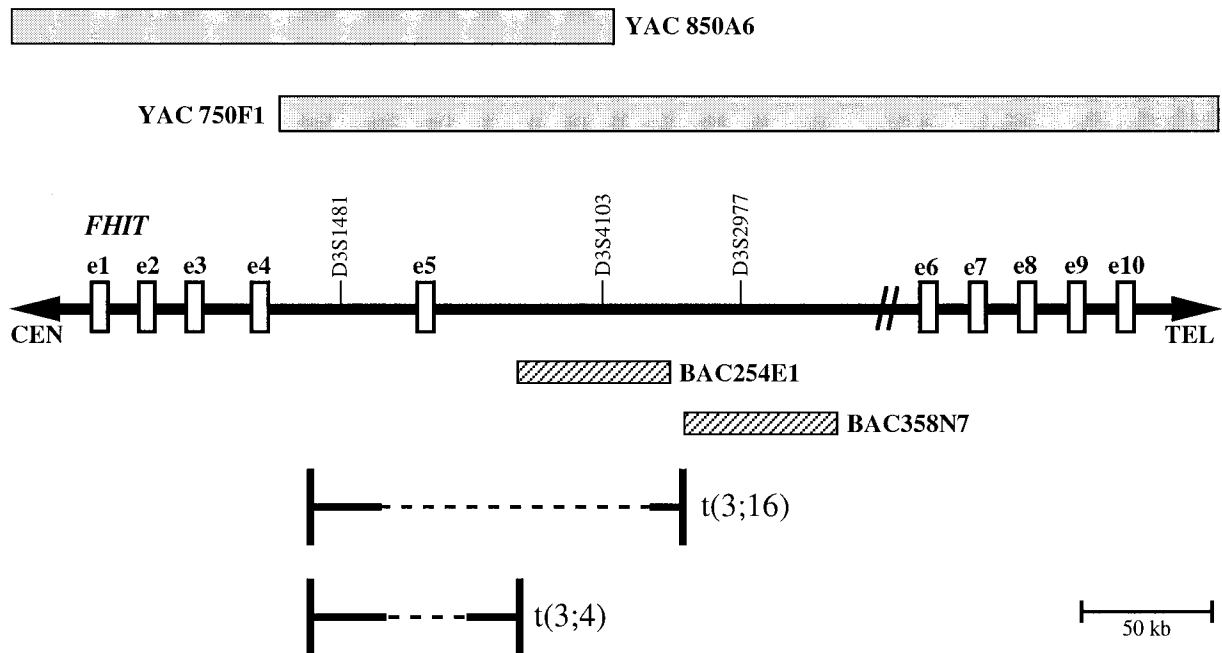


Figure 2. Map of the *FHIT* locus and position of fluorescence in situ hybridization probes used in this study. The relative positions of the translocation breakpoints are shown, with the deleted regions indicated by the dashed lines.

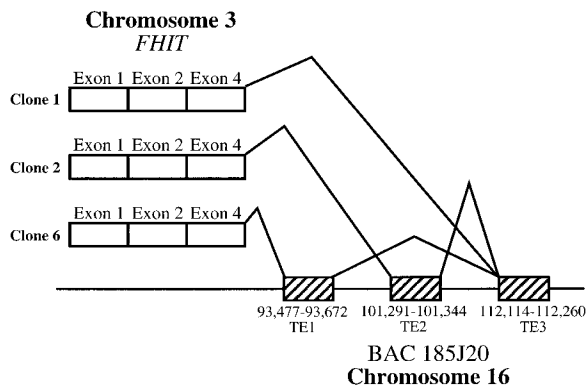


Figure 3. Results of 3' RACE from *FHIT* exon 1. Three transcripts were identified that contain *FHIT* exons 1, 2, and 4 and additional sequences corresponding to BAC 185J20 from chromosome region 16p13.3. The three transcripts appear to result from alternative splicing of the chromosome 16 cryptic exon sequences.

rather a nonfunctional transcribed and spliced region of chromosome 16 resulting from the translocation.

## DISCUSSION

We have shown that both homologs of chromosome 3 are involved in translocations with breakpoints in *FHIT*/FRA3B and at 4p16 and 16p13.3. All available data indicate that the two translocations are independent events, as opposed to a complex duplication of a single translocation event. The chromosome 3 breakpoints of both translocations are in the

region previously shown to be at the center of the FRA3B region, as determined by the position of aphidicolin-induced chromosome gaps on metaphase chromosomes (Smith et al., 1992; Wilke et al., 1994), and are accompanied by deletions encompassing *FHIT* exon 5. Using RT-PCR, a noncoding chimeric transcript was produced from the t(3;16) chromosome, which contained *FHIT* noncoding exons 1, 2, and 4 and three cryptic exon regions from 16p13.3. The RT-PCR results and FISH with whole-chromosome paint probes to chromosomes 3, 4, and 16 indicate that the t(3;16) was reciprocal. Chromosome 4 material was not identified on the other der(3) homolog by either FISH or RT-PCR. This finding suggests that the translocation involving 4p16 was nonreciprocal or, more likely, that the translocation involved only the telomeric region of chromosome 4 and was not detected by the paint probe.

It is now well established that large genomic deletions of up to hundreds of kilobases and aberrant transcripts are a common feature of mutations within the *FHIT* gene in a variety of tumors (Sozzi et al., 1998). The cells studied here were derived from an esophageal adenocarcinoma, a tumor type with increasing prevalence in the United States (Devesa et al., 1998). We have previously shown that *FHIT* deletions and aberrant transcripts are an early and common event in esophageal adenocarcinoma and in the precursor metaplastic tissue of

**FHIT exons 1, 2, and 4**

TCCCCGCTCTGCTCTGTCCGGTACAGGACTTTTGGCCCTCTGTTCCCGGGTCCCTCAGGCGGCCACCCAGTGGGCACACTCCCAGGCGGC  
 GCTCCGGCCCCGCGCTCCCTCCCTCTGCCTTTTCATTCCAGCTGTCAACATCCTGGAAGCTTTGAAAGCTCAGGAAAGAAGAGAAATCCACT  
 GAGAACAGTCTGTAAAGGTCCTAGTGTCTATCTACATCCAGACGGTGGAAAGGAGAGAAAGAGAAAGGATATCCTAGGAATACCTGCCT  
 GCTTAGACCCTCTATAAAAGCTCTGTGCATCCTGCCACTGAGGACTCCGAAAGGTTAGCAGTCTTCTGAAAGGGATATGAGGGACCTTTGG  
 GATCAGAGAGTGGATAGAGAAGACACAGATGGGATGAAGACTACTCTGGAAGGAGCATGGAGACCATAAAATGATGAGCCTGGGAAGTGTA  
 M M S L G S V  
 ↓ TE3  
 GACATCACTGATCACCTGGAAATGCACCTCTGTCTGTCTATAAGCAAGTGTGCATTTACGTGACCAATCTCTATTCCAACCTCTGGTATTG  
 D I T D H L E I A P L S A I S K C C I Y V T N L Y S N S G I  
 CAGTCTGCTTTGATATCCTGAGAAAGAGAAAGAAAGAAAGGAAAGTGAGAGNAAGAACAGGAGTCAGACTTATGGTCAGAGAACATTAGTG  
 A V C F D I L R R E E K K G K \*  
 GTGTTGTGAAATGCCTTTTAAAAATAATAAAGAGCTCATGCTCCTAATACTTCAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 4. Sequence of chimeric transcripts resulting from t(3;16) and identified by 3' RACE. The longest chimeric transcript containing TE1 and TE2 is shown. The 5' sequence (shaded) contains exons 1, 2, and 4 of *FHIT*. The 3' sequence contains cryptic exon sequences TE1 and TE3 (separated by the arrow) from 16p13.3. The amino acid sequence of the longest open reading frame is shown below the nucleotide sequence.

Barrett esophagus (Michael et al., 1997). The location of *FHIT* deletions in many tumors largely coincides with the location of *FRA3B*, as defined by chromosome gaps and breaks on metaphase chromosome preparations. Thus, it is likely that instability of the fragile site is mechanistically responsible for the deletions seen in *FHIT* in cancer cells. Additional support for this hypothesis comes from recent studies of other cloned fragile site regions. *FRA16D* (Mangelsdorf et al., 2000; Paige et al., 2000), *FRA7G* (Huang et al., 1996), and *FRA3B* (Arlt et al., unpublished observations) have been shown to contain internal deletions in cancer cells or cell lines. Thus, mutation by large deletion appears to be a common feature of common fragile sites in certain tumors.

In contrast to the large number of deletions seen in *FRA3B*, only one other tumor-associated translocation has been reported to occur in the *FHIT/FRA3B* region. This is a reciprocal t(3;12)(p14.2;q12) fortuitously found by examination of a chimeric transcript produced between the *HMGIC* gene and *FHIT* 3' exons (Geurts et al., 1997). The findings reported here suggest that these types of translocation events could be more common than is realized at present, since most investigations have looked for deletions in the *FHIT/FRA3B* region only by PCR or Southern blotting. There are many reported tumor karyotypes with breakpoints in the 3p14 region. The application of FISH or RT-PCR with *FHIT/FRA3B* probes and primers to the examination of these and other tumors would allow for a directed study to determine the

actual frequency of translocations or similar gross rearrangements.

Common fragile sites have been shown to be frequent sites for translocations in vitro after exposure of cultured cells to aphidicolin and folate stress (Glover and Stein, 1988; Wang et al., 1997). Together with the findings of fragile site-related deletions and instability in tumor cells, this suggests that instability at the fragile site is mechanistically responsible for the translocations reported here and for that shown by Geurts et al. (1997). In both cases, the translocations result in inactivation of the *FHIT* gene. We also found that both translocations in the BIC-1 cell line were accompanied by relatively large deletions of *FHIT* exon 5 and proximal intron 5 sequences. The temporal order and connection between the deletion and translocation events cannot be determined, but the *FHIT* gene could be inactivated by either the deletions or the translocations. As with previously reported *FHIT* deletions, instability at *FRA3B* could result in translocations and deletions that may confer a selective advantage for the cells. If the translocation and deletion events occurred sequentially, however, there would be no selection for loss of *FHIT* function during the latter event.

In summary, we have found both homologs of chromosome 3 to be translocated, with breakpoints in the *FRA3B* region, in an esophageal adenocarcinoma cell line. This finding lends support to early suggestions regarding common fragile sites and tumor-associated translocations. We suggest that

translocations involving FRA3B and other common fragile sites, some with the potential for forming chimeric transcripts with other genes, may be more frequent than is realized currently.

#### ACKNOWLEDGMENTS

We thank Robert Lyons for sequencing support, Cynthia Gaffney for assistance with manuscript preparation, and Diane Roulston for advice regarding nomenclature. This work was supported by National Cancer Institute grant CA43222 (TWG).

#### REFERENCES

- Arlt MF, Miller DE, Beer DG, Glover TW. Molecular characterization of FRAXB and comparative common fragile site instability in cancer cells. *Human Molecular Genetics*, submitted.
- Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs SC, Brown RS. 1979. Hereditary renal-cell carcinoma associated with a chromosomal translocation. *N Engl J Med* 301:592-595.
- Coquelle A, Pipiras E, Toledo F, Buttin G, Debatisse M. 1997. Expression of fragile sites triggers intrachromosomal mammalian gene amplification and sets boundaries to early amplicons. *Cell* 89:215-225.
- Dagenais SL, Guevara-Fujita M, Loechel R, Burgess AC, Miller DE, Yuzbasiyan-Gurkan V, Brewer GJ, Glover TW. 1999. The canine copper toxicosis locus is not syntenic with ATP7B or ATX1 and maps to a region showing homology to human 2p21. *Mamm Genome* 10:753-756.
- Devesa SS, Blot WJ, Fraumeni JF. 1998. Changing patterns in the incidence of esophageal and gastric carcinoma. *Cancer* 83:2049-2053.
- Gemmill RM, West JD, Boldog F, Tanaka N, Robinson LJ, Smith DI, Li F, Drabkin HA. 1998. The hereditary renal cell carcinoma 3:8 translocation fuses FHIT to a patched-related gene, TRC8. *Proc Natl Acad Sci USA* 95:9572-9577.
- Geurts JMW, Schoenmakers EFP, Roijer E, Stenman G, Van de Ven WJM. 1997. Expression of reciprocal hybrid transcripts of *HMGIC* and *FHIT* in a pleomorphic adenoma of the parotid gland. *Cancer Res* 57:13-17.
- Glover TW. 1998. Instability at chromosomal fragile sites. *Recent Results Cancer Res* 154:185-199.
- Glover TW, Stein CK. 1987. Induction of sister chromatid exchanges at common fragile sites. *Am J Hum Genet* 41:882-890.
- Glover TW, Stein CK. 1988. Chromosome breakage and recombination at fragile sites. *Am J Hum Genet* 43:265-273.
- Glover TW, Berger C, Coyle J, Echo B. 1984. DNA polymerase  $\alpha$  inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. *Hum Genet* 67:136-142.
- Hecht F, Glover TW. 1984. Cancer chromosome breakpoints and common fragile sites induced by aphidicolin. *Cancer Genet Cytogenet* 13:185-188.
- Huang H, Reed CP, Mordi A, Lomberg G, Wang L, Shridhar V, Hartmann L, Jenkins R, Smith DI. 1999. Frequent deletions within FRA7G at 7q31.2 in invasive epithelial ovarian cancer. *Genes Chromosomes Cancer* 24:48-55.
- Huang LC, Clarkin KC, Wahl GM. 1996. Sensitivity and selectivity of the DNA damage sensor responsible for activating p53-dependent G1 arrest. *Proc Natl Acad Sci USA* 93:4827-4832.
- Le Beau MM, Rowley JD. 1984. Heritable fragile sites and cancer. *Nature* 308:607-608.
- Mangelsdorf M, Ried K, Woollatt E, Dayan S, Eyre H, Finnis M, Hobson L, Nancarrow J, Venter D, Baker E, Richards RI. 2000. Chromosomal fragile site *FRA16D* and DNA instability in cancer. *Cancer Res* 60:1683-1689.
- Michael D, Beer DG, Wilke CW, Miller DE, Glover TW. 1997. Frequent deletions of *FHIT* and FRA3B in Barrett's metaplasia and esophageal adenocarcinomas. *Oncogene* 15:1653-1659.
- Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM, Huebner 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.
- Paige AJW, Taylor KJ, Stewart A, Sgouros JG, Gabra H, Sellar GC, Smyth JF, Porteous DJ, Watson JEV. 2000. A 700-kb physical map of a region of 16q23.2 homozygously deleted in multiple cancers and spanning the common fragile site FRA16D. *Cancer Res* 60:1690-1697.
- Rassool FV, McKeithan TW, Neilly ME, van Melle E, Espinosa R III, Le Beau MM. 1991. Preferential integration of marker DNA into the chromosomal fragile site at 3p14: an approach to cloning fragile sites. *Proc Natl Acad Sci USA* 88:6657-6661.
- Smith PP, Friedman C, Bryant EM, McDougall JK. 1992. Viral integration and fragile sites in human papillomavirus-immortalized human keratinocyte cell lines. *Genes Chromosomes Cancer* 5:150-157.
- Sozzi G, Huebner K, Croce CM. 1998. FHIT in human cancer. *Adv Cancer Res* 74:141-166.
- Wang L, Pardee W, Mullins C, Shridhar R, Rosati R, Wilke CM, Glover TW, Smith DI. 1997. Aphidicolin-induced FRA3B breakpoints cluster in two distinct regions. *Genomics* 41:485-488.
- Wilke CM, Guo SW, Hall BK, Boldog F, Gemmill RM, Chandrasekharappa SC, Barcroft CL, Drabkin HA, Glover TW. 1994. Multicolor FISH mapping of YAC clones in 3p14 and identification of a YAC spanning both FRA3B and the t(3;8) associated with hereditary renal cell carcinoma. *Genomics* 22:319-326.
- Yunis JJ, Soreng AL. 1984. Constitutive fragile sites and cancer. *Science* 226:1199-1204.