Frequent Breakpoints in the 3p14.2 Fragile Site, FRA3B, in Pancreatic Tumors1


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Abstract

FRA3B, at chromosomal band 3p14.2, is the most active common fragile site in the human genome. Homozygous deletions in the region of FRA3B have been observed in many types of solid tumors. Recently, the FHIT gene was reported to span FRA3B and was shown to be homozygously deleted in several gastric and colonic tumor cell lines. Several microsatellite markers that precisely define the 1.0-Mb region surrounding FRA3B and FHIT have been utilized, along with other 3p microsatellites, to analyze the loss of 3p sequences in 25 primary pancreatic adenocarcinomas. The high density of microsatellite markers in the 3p14.2 region enabled us to both identify losses within and flanking FRA3B in pancreatic cancer and define the breakpoints. We observed loss of heterozygosity of 3p14.2 markers in 16 of 25 pancreatic tumors and loss of heterozygosity of 3p markers outside of 3p14.2 in only 2 of 25 tumors of this type. There appears to be a dramatic clustering of chromosomal breakpoints at 3p14.2 in and immediately distal to FRA3B in pancreatic cancer. We detected no homozygous deletions in this region.

Introduction

Chromosomal regions in which gaps or breaks tend to occur when cells are exposed to specific conditions are known as fragile sites (1). Common fragile sites are those that occur in all individuals; some of these are localized to chromosomal bands that are frequently rearranged in various types of cancer (2). However, whether or not fragile sites play a causative role in these structural alterations has yet to be determined.

FRA3B, at chromosomal band 3p14.2, is the most highly inducible fragile site in the human genome (3), and its biological significance may be its involvement in several malignancies. Chromosomal breakpoints in the 3p13–p21 region encompassing FRA3B have been frequently observed in both small cell and non-small cell lung tumors (4–6), renal cell carcinoma (7), and breast cancer (8).

Adenocarcinoma of the pancreas is the fifth most common type of cancer in the United States (9). In the past three years, several chromosomal regions, including 6p, 6q, 8p, 9p, 1p, 17p, 18q, 21q, and 22q (10), have been implicated in the development and progression of pancreatic adenocarcinomas. However, loss of DNA sequences from chromosome 3p, commonly observed in many other solid tumors, has only rarely been observed in pancreatic cancer (11).

In this study, we report a high frequency of allelic loss (16/25) specifically within FRA3B, with minimal to no loss of polymorphic markers outside of FRA3B. The vast majority of chromosomal breakpoints detected occurred within the FRA3B region. We have observed homozygous deletions proximal to FRA3B in 2 of 25 primary pancreatic tumors, but none in the FRA3B region or in the FHIT gene which traverses this region. These results further support the claim that FRA3B is a hotspot for chromosomal changes leading to cancer.

Materials and Methods

Pancreatic adenocarcinomas and corresponding normal tissues were obtained from 25 patients treated at Harper Hospital, Detroit Medical Center (Detroit, MI) along with information on tumor stage, location, and differentiation. Of the 25 tumors analyzed, 10 were poorly differentiated, 5 were moderately differentiated, 2 were well differentiated, and for 2 cases no information was available. DNA was isolated from paraffin-embedded tissues as described previously (12).

Fifteen microsatellite markers from chromosome 3p were analyzed for genetic alterations. Twelve markers were obtained as MAPPAIRS from Research Genetics: D3S1577 (3p12.1), D3S1600 (3p14.1), D3S1312 (3p14.1), D3S1450 (3p14.2), D3S1481 (3p14.2), D3S1300 (3p14.2), D3S1313 (3p14.2), D3S1606 (3p21.1), D3S1029 (3p21.3), D3S1277 (3p23), D3S1560 (3p25.3), and D3S1270 (3p26). The remaining primer pairs were obtained from the Genome Database: D3S4260 (3p14.2), D3B1105223, D3S2977 (3p14.2, D3B451059), and PH13 (3p14.2, D3S4103).

PCR conditions were as follows: 50 ng of genomic DNA, 50 mM KCI, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2; 200 μM concentration of each primer; and 0.5 units of Taq polymerase (Promega) in a 12.5-μl reaction volume. One primer was end labeled using polynucleotide kinase and [γ-32P]ATP. The conditions for amplification were: 94°C for 5 min, then 30 cycles of 94°C for 30 s, 52-63°C for 30 s, and 72°C for 30 s in a Perkin Elmer-Cetus 4800 GeneAmp PCR system.

To detect homozygous deletions, we multiplexed two primer sets in one reaction. The primer sets D3S1577 and D3S1606, D3S1300 and D3S1600, D3S1029 and D3S1312, and D3S1277 and D3S1560 were multiplexed. These sets of primers had identical annealing temperatures and their products differed in size by about 50–100 bp. If in one sample, both sets of primers amplified a product from the normal DNA and only one primer set amplified a product from the corresponding tumor DNA, then it was scored as a homozygous deletion.

PCR products were processed by the addition of 5 μl of loading buffer consisting of 98% formamide, 1% EDTA (pH 8.0), 0.03% xylene cyanol, and 0.03% bromophenol blue. The reactions were denatured at 90°C for 2 min. Two μl were loaded onto a 6% polyacrylamide gel containing 50% urea for 2 to 3 h at 75 W. The gels were dried and exposed to X-ray film at −70°C.

Total RNA was extracted from the pancreatic tumor cell lines with Trizol (Life Technologies, Inc.) according to the manufacturer’s instructions. RT–PCR for the FHIT gene was performed exactly as described (14). The four pancreatic cell lines Panc1, Capan1, Capan2, and Cfpac1 were all obtained from American Type Culture Collection and maintained according to established conditions.

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1 The abbreviations used are: RT, reverse transcription; LOH, loss of heterozygosity; MIN, microsatellite instability.

4347
Results

Highly polymorphic markers from the short arm of chromosome 3 were chosen to test for LOH in 25 pancreatic adenocarcinomas. The markers were chosen to cover the entire short arm, with seven markers concentrated in the approximately 1-Mb region containing FRA3B. Both normal and tumor DNA from each patient were isolated from paraffin-embedded tissues. Three of the tumors (PT3, PT14, and PT68) showed MIN at most of the tested loci. An additional 10 tumors showed MIN in only 1–2 loci, with no apparent instability in the other 14–15 loci tested. Fig. 1 summarizes the results of the LOH and MIN observed in the 25 pancreatic tumors. Nine of 15 markers were highly informative (>70%), whereas the remaining 6 markers (D3S1577, D3S1312, D3S1480, D3S4260, D3S1311, and D3S1606) on average were 43% informative with our samples. The reported percentage of heterozygosity for these six markers ranged from 57 to 78% (Research Genetics). In spite of this, three sample pairs (samples 31, 70, and 75) were uninformative for 8 of 15 loci.

Three of the markers, D3S1577 (3p12.1), D3S1029 (3p21.3), and D3S1277 (3p23), showed no LOH in any of the tumors. The other 12 markers each detected LOH in one or more informative tumors. The battery of 15 markers detected LOH in a total of 16 of the 25 tumors. These results are displayed graphically in Fig. 2. Fig. 2 demonstrates much greater LOH in the 3p14.2 region than in any other chromosome 3p region tested. Fig. 3A illustrates some examples of markers showing LOH in a specific tumor, and Fig. 3B illustrates some examples of MIN. Two of the tumors (samples 49 and 74) showed LOH only for markers outside 3p14.2 (D3S1606 in 3p21.1 for tumor 49 and D3S1600 in 3p14.1 for tumor 74).

Homozygous deletions were not detected with any of the 3p14.2 markers in the primary pancreatic tumors. Nor were there any homozygous deletions with the FHIT gene using RT-PCR on the four pancreatic tumor cell lines. However, homozygous deletions were detected with the 3p14.1 marker D3S1600 in two tumor samples, specimens 23 and 74. To ensure that there was not an amplification problem with the DNA from these two samples, we multiplexed two sets of primers in one reaction, D3S1300 and D3S1600, and ran the amplification products on a 6% polyacrylamide gel. In sample pairs 23 and 74, both sets of primers amplified a product from the normal DNA, whereas only D3S1300 amplified a product from the corresponding tumor DNA (Fig. 3C). The inability to amplify a product using the D3S1600 marker within the same reaction that amplified a product using the D3S1300 marker lead us to believe that both copies of the D3S1600 locus are deleted in the two tumor samples. We also multiplexed primer sets D3S1577 and D3S1606, D3S1029 and D3S1312, and D3S1277 and D3S1560, but did not observe any homozygous deletions with these markers.

The microsatellite markers also enabled us to determine chromosome 3p breakpoints in the tumors. The high density of markers in the 1-Mb region surrounding FRA3B enabled us to precisely localize the breakpoints between these markers and to position the breakpoints relative to the FRA3B region. Each of the tumors were scored for the number of breakpoints on chromosome 3 and the number of breakpoints in the FRA3B region. A marker that exhibited LOH and was flanked by two informative markers that did not show LOH was scored as two breakpoints, one on each side of the loss. For example, sample 13 had five detectable breakpoints on chromosome 3p, and three of these breakpoints occurred in the FRA3B region. Another specimen, sample 64, had four detectable 3p breakpoints, and all four were within FRA3B. Fig. 4 illustrates the positioning of the breakpoints within chromosome 3p based on the LOH data gathered from the 25 pancreatic tumors. The positioning of the breakpoints (Fig. 4, arrows) clearly demonstrates a hotspot for chromosomal breakage within the FRA3B region. The end points of the raised lines (Fig. 4), as indicated by the specific markers, represents the boundaries of the region where additional breakpoints occur. The precise localization of the breakpoints within these regions could not be delineated because of the presence of flanking uninformative markers (see Fig. 1). Of the 40 breakpoints mapped by this analysis, 32 (80%) fall within the 1-Mb region of the sequence containing FRA3B.

Discussion

Pancreatic cancer is highly aggressive and the fifth most common cancer in the United States. Little is known about the molecular pathways that underlie the development of pancreatic cancer. Karyotypic and microsatellite analyses of pancreatic cancers have revealed a spectrum of common chromosomal changes (10, 11, 13). The frequency of chromosome 3p loss has ranged from 13% in a report by Griffin et al. (11) to as high as 40% in a report by Hahn et al. (13). However, in the report by Hahn et al. (13), they tested the Research Genetics Genethon marker set II. This marker set dispersedly covered the short arm of chromosome 3 (about one marker for every 10 cM).
These markers did not cover the FRA3B region and did not include any of the markers used in this study. In addition, they did not observe a clustering of breakpoints in any specific region of chromosome 3p.\(^4\)

Our results confirm that loss of 3p DNA sequences outside of the FRA3B region is a relatively uncommon event in pancreatic cancer, occurring in only 2 of the 25 tumors examined (8%). However, breakpoints in the FRA3B region were detected in 16 of 25 (64%) of the tumors analyzed. Several of the samples had multiple breakpoints in the FRA3B region, with no detectable loss of chromosome 3 sequences outside of this region. Thus, chromosomal breakage and loss in the FRA3B region appears to be a frequent event in pancreatic cancer.

Recently, the fragile histidine triad (FHIT) gene was shown to span the hereditary renal cell carcinoma translocation breakpoint and FRA3B (14). Segments of this gene were shown to be homozygously deleted in stomach and pulmonary lung tumor cell lines as well as in primary lung tumors (14, 15). We analyzed the FHIT gene using RT-PCR in a total of four pancreatic tumor cell lines (Panc1, Capan1, Capan2, and Cfpacl; all cell lines were obtained from American Type Culture Collection and maintained according to established conditions). In all four cell lines, a single, prominent RT-PCR product, with no major aberrant transcription products, was observed (data not shown). This clearly indicated to us that the FHIT gene was not homozygously deleted in these tumor cell lines.

Although we have observed frequent deletions and breakpoints in the FRA3B region, as scored by loss and retention of flanking polymorphic markers, we have not detected any homozygous deletions within this region in any of the primary pancreatic tumors. However, we did detect a homozygous deletion of the 3p14.1 locus D3S1600 in two tumors, samples 23 and 74.

While scoring for LOH in these tumors, we observed extensive MIN in samples 3, 14, and 68, in which at least 5 of the 15 markers showed MIN. There were an additional 10 tumors that showed MIN with only one to two markers. The MIN was often expressed as single bands rather than as a ladder of new alleles, as often seen in the hereditary nonpolyposis colorectal tumors (16, 17). The frequency of MIN in our pancreatic tumors is roughly comparable to that reported in pancreatic tumors by other groups (18).

When we positioned the chromosome 3p breakpoints based on our LOH studies, we observed a dramatic clustering within FRA3B (Fig. 4). It would therefore seem that FRA3B is a hotspot for chromosomal breakage in pancreatic cancer. Also indicated in Fig. 4 is a cosmid (c55), which appears to define the middle of the FRA3B region (19), and two regions flanking c55, which defined clusters of aphidicolin-induced breakpoints generated in somatic cell hybrids (20). The breakpoints observed in the pancreatic tumors appear to be slightly

\(^4\) S. Kern, personal communication.
different cancers. FRA3B is only one of many common fragile sites
Wilke et al. (19).

distal to the epicenter of aphidicolin-induced breakage observed by
FRA3B may be common events during the development of many
different cancers. FRA3B is only one of many common fragile sites
(21, 22). One could speculate that instability at a number of these
loci may occur during cancer development. Genes in the vicinity of these
loci, as FHT is for FRA3B, may be a whole new group of mutually
targets that participate in cancer development and progression.

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References

1. Sutherland, G. R., and Hecht, F. Fragile sites on human chromosomes. Oxford
2. Yunis, J. J., and Sorensen, P. A. Constitutive fragile sites and cancer. Science (Wash-
3. Smeets, D. F. C. M., Scheres, J. M. J. C., and Hustinx, T. W. J. The most common
4. Daly, M. C., Douglas, J. B., Bleheen, N. M., Hasleton, P. S., Twemlow, P. R.,
Sunderston, V., Carritt, B., Bergh, J., and Rabbits, P. H. An unusually proximal
deletion on the short arm of human chromosome 3 in a patient with small cell lung
Papenema, S., and Buys, C. H. M. Deletion of a DNA sequence at the chromosomal
region 17q21 in familial breast cancer and ovarian cancer involves the wild type
Redston, M. S., Caldas, C., Weinstein, C. L., Fischer, A., Ayo, J. C., Ruban, R. H.,
region 17q12–21 in familial breast cancer and ovarian cancer involves the wild type
11. Heim, S. Interstitial deletion of the short arm of chromosome 3 as a primary
chromosome abnormality in carcinomas of the breast. Genes Chromosomes & Can-
7–26, 1993.
13. Volovitz, A. B., Ruban, R. H., Redston, M., Caldas, C., Powell, S. M., Kinzler, K. W.,
region 17q12–21 in familial breast cancer and ovarian cancer involves the wild type
instability in pancreatic cancer and poorly differentiated type of gastric cancer.
17. Wilke, C. M., Hall, B. K., Hoge, A., Pardee, W., Smith, D. L., and Glover, T. W.
FRA3B extents over a broad region and contains a spontaneous HPVI6 integration
site: direct evidence for the coincidence of viral integration sites and fragile sites.
19. Heim, S. Interstitial deletion of the short arm of chromosome 3 as a primary
chromosome abnormality in carcinomas of the breast. Genes Chromosomes & Can-
20. Pardee, W., Wilke, C. M., Wang, L., Shridhar, R., Mullins, C. M., Hoge, A., Glover,
524–531, 1996.
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