Advances in Brief

Allelic Loss and Mutational Analysis of the DPC4 Gene in Esophageal Adenocarcinoma

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Abstract

DPC4, a recently cloned gene located on 18q21.1, is inactivated in almost one half of pancreatic adenocarcinomas. To determine whether DPC4 inactivation is involved in esophageal adenocarcinoma, we have analyzed aneuploid populations from biopsies of 35 patients with Barrett’s esophagus who had premalignant epithelium, adenocarcinoma, or both. Sixteen of 35 patients (46%) had allelic loss at 18q21.1, including 7 patients who had only premalignant tissue present in their Barrett segment. In addition, three of four patients (75%) with 18q21.1 loss in their aneuploid populations had the allelic loss present in diploid cells. Mutational analysis of DPC4 did not reveal any inactivating alterations in the gene. These data indicate that allelic losses at 18q are selected during neoplastic progression in Barrett’s esophagus, but the targeted gene remains to be identified.

Introduction

Allelotype analysis of esophageal adenocarcinoma has shown previously that allelic loss of 18q occurred in approximately 43% of tumors (1). The prevalence of 18q allelic losses was significantly above the background level of allelic loss in these cancers, suggesting the selected inactivation of a tumor suppressor gene. The recent identification of DPC4 (deleted in pancreatic carcinoma, locus 4) on 18q21.1 as a genetic target in pancreatic carcinomas stimulated us to investigate the involvement of this locus in esophageal adenocarcinoma (2).

We screened flow-sorted aneuploid populations from 41 patients with Barrett’s esophagus who had premalignant epithelium, cancer, or both using polymorphic markers that are tightly linked to the DPC4 locus. Thirty-five patients were informative for at least one of the 18q21.1 polymorphic markers that were used in this study. In addition, Ki67-positive diploid epithelial cells were available for analysis from a subset of these patients. Using these polymorphic markers, we determined the prevalence of the 18q21.1 allelic losses and DPC4 alterations in esophageal adenocarcinoma and investigated the relationship of these events to the development of aneuploidy and cancer during neoplastic progression.

Materials and Methods

Patient Samples. Tissue samples were obtained from surgical specimens and endoscopic biopsies using our previously published mapping techniques which permit the localization of abnormalities within the esophageal mucosa (3, 4). In addition, normal gastric tissue was obtained from each patient. Histological classification was determined as described previously (3). Aneuploid populations and the corresponding diploid populations from each biopsy were isolated using DNA content flow cytometric cell sorting as described previously (3, 4). Ki67/DNA content multiparameter flow cytometric cell sorting was used to purify populations of proliferating diploid epithelial cells according to our published protocols (3, 4). DNA from normal and flow-sorted samples was amplified using the technique of whole-genome amplification according to published protocols (1, 5). Aliquots from each reaction were then used as the source of material for subsequent locus-specific analysis.

PCR and Sequencing. Microsatellite analysis was performed on whole-genome amplified material as described previously (1, 5). Fluorescence-based sequencing reactions were done with PCR products obtained directly from 10,000 flow-sorted cells according to our published methods. Primers for amplifying and sequencing DPC4 were based on sequences available on the Internet (http://www.med.jhu.edu/pancreas/index.htm).

Results and Discussion

The progression to cancer in patients with Barrett’s esophagus is characterized by loss of cell cycle regulation, accumulation of multiple genetic abnormalities, and the appearance of aneuploid cell populations (1, 4, 6). To investigate the potential involvement of the DPC4 locus in esophageal adenocarcinoma, we screened 41 patients with Barrett’s esophagus who had aneuploid cell populations in premalignant tissue, cancer, or both for allelic loss at 18q21.1. Flow-sorted aneuploid and the corresponding normal tissues were available from these 41 patients. Thirty-five patients were informative for at least one of the 18q21.1-specific markers (D18S46, D18S479, and D18S474) used in this study (7). Aneuploid populations from 16 of these patients (46%) had allelic loss involving 18q21.1 (Fig. 1 and Table 1). There were 12 cases of allelic loss in which the patient was also informative for an 18p-specific marker, D18S53. In nine of these cases (75%), the allelic loss involved only the q arm of chromosome 18, indicating that the majority of losses detected in this study represented subchromosomal events such as might be mediated by mitotic recombination or interstitial deletions rather than nondisjunction of an entire chromosome. The 18q allelic losses were detected in aneuploid populations of 10 of 22 cancers (45%) and in 6 of 13 patients (46%) who had only premalignant epithelium without cancer.

In our previous allelotype of esophageal adenocarcinoma, we detected 18q allelic loss (D18S34; 18q12.2-12.3) in 6 of 14 informative patients (43%), with a background level of allelic losses of 23% (1). Statistical analysis indicated that these 18q losses had a P of between 0.05 and 0.10 of occurring at random, i.e., 0.05 < P < 0.10. In the present study, 18q allelic losses were detected in aneuploid cell populations of 16 of 35 patients (46%) with P = 0.003, for a one-sample test of a proportion, of being equal to the background frequency of 0.23. Therefore, these events appear to be highly selected during neoplastic progression in Barrett’s esophagus.

Previous studies have suggested that mutations in the DPC4 cluster within exons 8 and 11 of the gene (2, 8–12). This region contains a high level of homology between DPC4 and the Drosophila melanogaster Mad and Caenorhabditis elegans Sma genes. Sufficient material for direct sequencing of this region was available from 12 of the 16 patients with 18q allelic loss in aneuploid cell populations. In all
cases, the sequences obtained for exons 7 through 11 of DPC4 were identical to those in matching normal tissue from the same patient.

The DPC4 gene is inactivated by homozygous deletion in approximately 30% of pancreatic adenocarcinomas (2). However, homozygous deletions and allelic losses could be obscured by contaminating genetically normal stromal cells (1, 13). Therefore, we used flow cytometric cell sorting, which can purify aneuploid cell populations such as those evaluated in this study to 99% or greater homogeneity (data not shown), to purify cells for analysis of homozygous deletions and allelic losses. Using polymorphisms that have been shown to be tightly linked to the DPC4 locus and to be included in the majority of homozygous DPC4 deletions that have been reported in pancreatic adenocarcinoma, we found at least one allele in each highly purified aneuploid cell population from the 41 patients in this study, suggesting that 18q21.1 homozygous deletions are not frequent abnormalities in Barrett’s esophagus. Furthermore, the PCR template for sequencing DPC4 was obtained from all samples with 18q21.1 allelic loss that were assayed for mutation.

Previous studies of biopsy samples have used retention of heterozygosity in a region of allelic loss as a marker for homozygous deletions (14). We considered the possibility that even less than 1% contamination by normal cells might result in the apparent retention of heterozygosity and evaluated flanking markers for allelic loss. However, there was no evidence for retention of heterozygosity at the DPC4 locus in any of the 22 patients who were informative for at least two of the 18q21.1-linked markers in this study, further suggesting that homozygous deletions of DPC4 are not common in Barrett’s esophagus or esophageal adenocarcinoma.

Mutational screening of DPC4 in a variety of cancers has suggested that inactivation of DPC4 may be limited to those of the pancreas and possibly other tissues of the gastrointestinal tract (10). It is interesting to note that pancreatic and esophageal adenocarcinomas share similarities at the molecular level. For example, both cancers have high prevalences of 17p allelic loss and p53 inactivation, allelic loss at 9p21 with inactivation of CDKN2/p16, and an absence of mutations in APC (adenomatous polyposis coli; Refs. 1, 6, 13, and 15). However, pancreatic adenocarcinomas have a higher prevalence of 18q21.1 allelic loss, 90% compared to 46% in esophageal adenocarcinoma, and contain mutations or homozygous deletions of DPC4 in approximately 50% of the samples reported (2, 7). Therefore, our results suggest that the tissue distribution of DPC4 inactivation may be limited to a subset of gastrointestinal cancers.

Six of the patients with 18q21.1 allelic loss in their aneuploid cell populations had only premalignant tissue present in their Barrett’s segment (Table 1). Furthermore, in one of two patients from whom both premalignant tissue and cancer were available for analysis, the 18q21.1 allelic loss detected in a region of cancer was also present in the premalignant epithelium. Thus, 18q21.1 allelic losses were detected in aneuploid cell populations in 7 of 15 patients (47%) in whom premalignant samples were evaluated in this study. Flow-sorted Ki67-positive diploid epithelial cells were available for analysis from four of the patients with 18q21.1 loss in aneuploid populations in premalignant epithelium. In three of these four patients (75%), 18q21.1 allelic losses were detected in the diploid cell populations, whereas in the fourth patient, we detected the allelic loss and aneuploidy simultaneously (Table 2). In every case the same 18q21.1 allele was lost in diploid and aneuploid cell populations. Therefore, our data suggest that 18q allelic losses can occur as early events in Barrett’s epithelium, which can precede the development of aneuploidy and cancer. However, the targeted gene on 18q in esophageal adenocarcinoma remains unknown, and additional work will be required to determine whether candidates such as DCC (deleted in colorectal carcinoma) or as yet identified genes are involved (8, 16). In addition, further study will be required to determine the relationship of 18q allelic loss to other events of neoplastic progression in Barrett’s esophagus, including allelic losses at 17p, 9p, 5q, and 13q and increases in G1, S, and 4N (G2/tetraploid) fractions (1, 4, 6).

References

Table 1 Prevalence of 18q21.1 allelic losses

<table>
<thead>
<tr>
<th>Grade</th>
<th>n</th>
<th>Allelic loss/informative patient (%)</th>
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<tbody>
<tr>
<td>Premalignant</td>
<td>16</td>
<td>6/13 (46)</td>
</tr>
<tr>
<td>Cancer</td>
<td>25</td>
<td>10/22 (45)</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>16/35 (46)</td>
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Table 2 Order of 18q21.1 allelic losses

<table>
<thead>
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<th>Patient</th>
<th>Grade</th>
<th>Ploidy</th>
<th>Loss of heterozygosity</th>
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<tbody>
<tr>
<td>12514</td>
<td>Premalignant</td>
<td>2.0N</td>
<td>–</td>
</tr>
<tr>
<td>15063</td>
<td>Premalignant</td>
<td>2.0N</td>
<td>+</td>
</tr>
<tr>
<td>130N</td>
<td>Premalignant</td>
<td>2.0N</td>
<td>+</td>
</tr>
<tr>
<td>126N</td>
<td>Premalignant</td>
<td>2.0N</td>
<td>–</td>
</tr>
</tbody>
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Fig. 1. A, 18q21.1 allelic losses in aneuploid cell populations from three patients with Barrett’s esophagus (D18S474: Lanes 1, 2, 5, and 6; D18S46, Lanes 3 and 4). Lanes 1, 3, and 5: constitutive tissue; Lanes 2, 4, and 6: aneuploid cells. B, 18q21.1 allelic loss (D18S46) in G, diploid and aneuploid cell populations. Lane 7, constitutive tissue; Lanes 8-10, G, diploid populations; and Lane 11, aneuploid population from the same patient (126N).
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