Allelotype Analysis of Esophageal Adenocarcinomas: Evidence for the Involvement of Sequences on the Long Arm of Chromosome 4

Zane T. Hammoud, Zahid Kaleem, Joel D. Cooper, R. Sudhir Sundaresan, G. Alexander Patterson, and Paul J. Goodfellow

Department of Surgery, Divisions of General Surgery [Z. T. H., P. J. G.] and Cardiothoracic Surgery [J. D. C., R. S. S., G. A. P.] and Department of Pathology [Z. K.]. Washington University School of Medicine, St. Louis, Missouri 63110

ABSTRACT

The incidence of esophageal adenocarcinoma has increased dramatically over the past 20 years. The causes for this change in incidence and the genetic defects that underlie tumorigenesis are unknown. We performed loss of heterozygosity (LOH) studies in esophageal adenocarcinomas in an effort to map the location of tumor suppressor genes involved in the initiation or progression of this cancer. A genome-wide search for LOH was undertaken using microsatellite repeat polymorphisms and a panel of 27 tumor and matched normal DNAs.

This is the first report of an allelotype analysis of esophageal adenocarcinomas. We observed frequent loss of sequences on the short arm of chromosome 17 in the region of the TP53 gene. We also identified a region on 4q lost in more than half of the tumors investigated. The high rate of LOH for 4q sequences speaks to the involvement of an as yet unidentified tumor suppressor gene in esophageal adenocarcinoma tumorigenesis.

INTRODUCTION

In 1976, Nowell hypothesized that cancers develop as a consequence of acquired genetic instability that predisposes to the evolution and multiplication of abnormal clones of cells with accumulated genetic errors (1). Since that time, this hypothesis has been refined, and it has been demonstrated that some types of cancer do indeed develop as the result of accumulation of mutations in oncogenes and tumor suppressor genes (2). Deletions of tumor suppressor genes occur frequently in human malignancies. Such events can be detected using markers from the region of the genome that includes a tumor suppressor gene. LOH studies serve to identify deletions in cancer cells and by doing so point to the locations of tumor suppressor genes.

Esophageal adenocarcinoma has become one of the fastest-growing cancers over the past 20 years (3). The strong association between esophageal adenocarcinoma and the presence of columnar epithelium in the esophagus has remained a consistent finding since Barrett first described the condition in 1950 (4). The precise cause of this metaplastic change from squamous to columnar epithelium, commonly referred to as Barrett’s esophagus, remains uncertain. It is widely held, however, that chronic gastroesophageal reflux disease underlies this metaplasia.

The genetic events that lead to the development of esophageal adenocarcinoma have yet to be elucidated. Studies conducted thus far have focused on known tumor suppressor genes, particularly TP53 on chromosome 17p (5). There is additional evidence that multiple genetic abnormalities contribute to the development of this cancer (6).

We undertook a comprehensive allelotype study of esophageal adenocarcinomas to detect genetic events that might contribute to the initiation and/or progression of these cancers. Genotyping studies were performed using highly polymorphic markers from every chromosomal arm with the exception of the short arms of the acrocentrics and the sex chromosomes. LOH studies serve to identify regions that are deleted frequently and by doing so point to the location of tumor suppressor gene(s) important in the disease process.

MATERIALS AND METHODS

Tissue Samples and DNA Extraction. DNA was prepared from archival pathology specimens from 30 patients treated surgically for esophageal adenocarcinoma at the Washington University Medical Center (St. Louis, MO) between 1986 and 1995. The existing H&E-stained slides were reviewed to confirm the diagnosis of adenocarcinoma and to select blocks that included tumor tissues with high neoplastic cellularity. Serial sections from the tissue blocks of interest were prepared and mounted on slides. Areas of >70% neoplastic cell content were identified (by assessing the histology of representative stained slides) and marked on the unstained slides, and the tissues were microdissected. Normal (nonneoplastic) genomic DNA was prepared from either uninvolved lymph nodes or gastric tissue. Standard methods were used for DNA extractions (7, 8).

DNA Marker Genotyping Studies. Tumor DNAs were investigated for LOH using the polymorphic microsatellite markers listed in Table 1. PCR primers were either purchased from Research Genetics (Huntsville, AL) or synthesized using published sequences. The reaction conditions for PCR amplification and methods for detection of the markers were as described previously by our group (9).

For all informative cases, LOH was categorized as ≥67% reduction in intensity of one allele in the tumor DNA compared to the corresponding allele in the normal DNA from the same patient. All genotyping reactions were interpreted independently by Z. T. H. and P. J. G. In those instances in which there was a subtle alteration in the relative intensity of alleles, the reactions were repeated and the pattern of allelism evaluated by scanning densitometry (Computer Eyes/RT SCSI Video Frame Grabber Analysis system: Digital Vision, Inc.). Tumors exhibiting a reduction in intensity of alleles of more than 25% but less than 67% were categorized as showing AI for the marker under investigation.

RESULTS

A total of 30 paired normal-tumor DNA samples from patients with esophageal adenocarcinoma were tested initially for LOH using 39 markers. Two tumor DNA samples revealed MI with multiple markers. They exhibited the RER phenotype and were therefore excluded from our LOH analysis. One tumor DNA sample failed to produce a PCR product for multiple assays despite several extraction attempts; this sample was also eliminated from the LOH analysis. Table 1 lists the markers used, their map positions, the number of informative cases, and the observed frequency of LOH at each locus. Representative autoradiographs showing LOH, AI, and retained heterozygosity are shown in Fig. 1.

LOH was observed in one or more tumor specimens for 38 of the 39 chromosomal arms investigated. The short arm of chromosome 10 was the only region for which no LOH was identified (21 informative cases). The observed frequency of LOH ranged from 5% for the 11p marker to 63% for the marker on 17p. The mean observed frequency

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1 To whom requests for reprints should be addressed, at Department of Surgery, Box 8109, Washington University School of Medicine, 660 South Euclid, St. Louis, MO 63110. Phone: (314) 362-8106; Fax: (314) 362-8620; e-mail: goodfellow@wudos2.wustl.edu.

2 The abbreviations used are: LOH, loss of heterozygosity; AI, allelic imbalance; RER, replication error; FAL, fractional allelic loss; MI, microsatellite instability.

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of LOH was 20.4% (SD, 13). Three regions showed very frequent LOH: 17p, 4q, and 18q (63%, 54%, and 40% respectively). The percentage of LOH observed for each chromosomal arm is presented in graphical form in Fig. 2.

The number of LOH events and the regions showing loss varied among the tumors (Table 2). Only one specimen (tumor 2), which was informative for 31 chromosomal regions, showed no evidence of LOH. For our collection of tumors and the markers we investigated, the FAL ranged from 0.02 to 0.53, and the mean FAL was 0.20. Table 2 shows the FAL for each sample as well as the chromosomal arms exhibiting LOH.

**DISCUSSION**

We undertook an allelotype analysis of esophageal adenocarcinoma in an effort to map the location of tumor suppressor genes that play a role in the development of this cancer type. Frequent deletion of a specific chromosomal region in a given cancer type often reflects the involvement of a tumor suppressor gene. Previous allelotype studies of esophageal cancers have focused largely on squamous cell carcinomas (10). LOH studies in esophageal adenocarcinomas have been restricted to the investigation of chromosomal regions known to contain tumor suppressor genes (5, 6, 11–13). We used one marker from each chromosomal arm (with the exception of the short arms of the acrocentrics and the sex chromosomes). A subset of the markers we investigated was selected based on proximity to known tumor suppressor genes (Table 1).

Two chromosomal regions, 17p and 4q, showed a significantly increased frequency of LOH. Regions showing a frequency of LOH greater than two SDs above the mean are likely to include tumor suppressor genes that contribute to the cancer phenotype. The highest frequency of LOH observed in this study was with the 17p marker, which maps near the TP53 gene. The frequent loss of 17p sequences we observed is consistent with previous reports of LOH in esophageal adenocarcinomas (3) and likely reflects the involvement of TP53. The second most frequent site of LOH observed was on 4q (55%). Genotyping our tumor collection with two additional 4q markers confirmed the observation that 4q is deleted frequently. Furthermore, these results indicated that only a region of 4q was lost in the majority of the tumor specimens (Fig. 3). According to the Cooperative Human Linkage Center’s latest sex-averaged linkage map of chromosome 4, D4S193, the most proximal marker, is located in a region on 4q that is 120–128 cM from pter; similarly, D4S2374 is located at 203 cM, and D4S171 is located at 241 cM from pter. These preliminary data indicate that the region of loss spans a distance of at least 40 cM located on distal 4q. Loss of sequences on the long arm of chromosome 4 has not been reported previously for esophageal adenocarcinomas. 4q LOH was not correlated with patient age, tumor grade, or tumor stage in our patient series (data not shown).

LOH on 4q has been reported for a number of other tumor types. Mitra et al. (14) reported frequent loss of 4q sequences in cervical cancers; this group found a common region of deletion encompassing the region 4q21–q25. Polascik et al. (15) found distinct regions of loss on 4q in bladder cancers; they reported a 14 cM area of loss on distal 4q between markers D4S426 and D4S408, a region that is contained within the one we report here. Both groups speculated as to the existence of a tumor suppressor gene on 4q. There have been a number of additional reports on 4q LOH in a variety of tumors (16–18).

In our study, several markers revealed a moderately high frequency of LOH. A frequency of greater than 30% was observed with the 8p, 9q, 10q, 18q, and 19p markers investigated (Fig. 2). LOH on 8p and 9q is a feature of many cancers (16, 19–21). Peiffer et al. observed a 40% LOH at 10q in endometrial carcinoma and suggested the existence of a putative tumor suppressor gene at 10q23–q26 (9). 18q is known to contain the DCC gene, and a role for this gene in esophageal adenocarcinomas has been suggested previously (6). The DPC4 gene (22), which maps close to DCC on 18q, might also be involved in esophageal adenocarcinomas. 19p LOH has been observed in cervical carcinoma (23), in glioma (24), and in ovarian cancer (25). The frequency of LOH observed for 8p, 9q, 10q, 18q, and 19p in this study may indicate the involvement of tumor suppressor genes in those regions.

In addition to LOH, MI and AI were found during the course of our genotyping studies. Widespread MI (the RER phenotype) was seen in two tumor DNA samples, a finding consistent with some underlying defect in DNA mismatch repair in these samples. Other investigators have reported on RER in esophageal adenocarcinomas (26). Although MI at multiple marker loci was observed only in these two specimens,
21 of the 27 tumor DNA samples we investigated exhibited MI at one or two marker loci. The majority of MI identified was seen with seven of the markers. The markers for 5p, 7q, 8q, 13q, 4q, and 22q each revealed two instances of MI, and the 18q marker revealed three cases of MI. A low frequency of MI has been documented previously for esophageal adenocarcinomas (27).

AI was noted for a number of tumor DNA samples in this study. AI could result from contamination of neoplastic cellular DNA with normal cellular DNA. Our specimens were selected carefully and microdissected to enrich for neoplastic cellularity. LOH was readily evident in most tumor DNA samples. Therefore, it seems unlikely that the observed AI results from admixture of neoplastic and normal cellular DNA. Another possible explanation for AI would be duplication of a chromosome or of a chromosomal region. Alternatively, a deletion occurring late in the clonal expansion of a tumor would result in a pattern of allelism that would appear as AI. There were relatively

Table 2  Regions lost and FAL for individual tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>No. informative arms</th>
<th>Regions lost</th>
<th>FAL (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>1p, 8p, 8q, 17p, 19p</td>
<td>5/32 (15.6)</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>6p, 6q, 7q</td>
<td>17/32 (53.1)</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>1p, 1q, 3p, 3q, 5p, 6p, 8q, 10q, 12p, 12q, 13q, 17p, 17q, 18p, 19p, 19q, 21q</td>
<td>6/23 (26.1)</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>5q, 7q, 10q, 12q, 13q, 17p, 17q, 18p, 19p, 19q, 21q</td>
<td>4/28 (14.3)</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>1p, 8p, 9p, 17p</td>
<td>3/27 (11.1)</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>4q, 6q, 7q, 10q, 11q, 12p, 14q, 17p, 17q, 18p, 18q</td>
<td>1/28 (3.7)</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>1q, 9q, 17p</td>
<td>0/31 (0)</td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>2q, 6q, 10q, 11q, 12p, 16p, 17p, 19p, 19q, 21q</td>
<td>17/33 (51.5)</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>9p, 14q, 17p, 22q</td>
<td>12/28 (42.9)</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>4q, 6q, 7q</td>
<td>8/33 (24.2)</td>
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<tr>
<td>11</td>
<td>23</td>
<td>2p, 2q, 3p, 3q, 5p, 9q, 14q, 17q</td>
<td>8/30 (26.7)</td>
</tr>
<tr>
<td>12</td>
<td>33</td>
<td>2q, 4q, 4q, 6q, 6q, 9q, 9q, 16q, 17q, 18q, 22q</td>
<td>2/31 (6.5)</td>
</tr>
<tr>
<td>13</td>
<td>30</td>
<td>5p, 4q, 6q, 6q, 9q, 11q, 17p, 17q</td>
<td>1/35 (2.9)</td>
</tr>
<tr>
<td>14</td>
<td>31</td>
<td>3p, 4q, 4q</td>
<td>6/26 (23.1)</td>
</tr>
<tr>
<td>15</td>
<td>35</td>
<td>17p</td>
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<tr>
<td>16</td>
<td>26</td>
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</tr>
<tr>
<td>17</td>
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<td>18</td>
<td>30</td>
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<tr>
<td>19</td>
<td>20</td>
<td>3p, 4q, 5p, 6q, 6q, 9q, 10q, 12p, 12q, 18q, 19q</td>
<td>10/26 (39.6)</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>4q, 7q, 13q, 15q, 16q, 22q</td>
<td>4/24 (16.7)</td>
</tr>
</tbody>
</table>

* Mean FAL = 20.6.

Fig. 2. Summary of frequencies of LOH by chromosomal arm. Markers investigated are listed in Table 1. The number of specimens informative for any marker ranged from 11 to 24.
Fig. 3. Patterns of 4q deletion in 27 esophageal adenocarcinomas. O, retained heterozygosity; •, LOH; NI, noninformative specimen; MI, microsatellite instability; AI, allelic imbalance. The approximate location of the markers is indicated in parentheses.

**REFERENCES**

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