Refrinement of Regions with Allelic Loss on Chromosome 18p11.2 and 18q12.2 in Esophageal Squamous Cell Carcinoma

Jayaprakash D. Karkera, 2 Saleh Ayache, 2 Rodman J. Ransome, Jr., Marvin A. Jackson, Ahmed F. Elsayem, Rajagopalan Sridhar, Sevilla D. Detera-Wadleigh, and Robert G. Wadleigh 3

Medical Oncology Section, Department of Veterans Affairs Medical Center, Washington, DC 20422; Departments of Pathology [R. S., M. A. J.], Medicine [A. F. E.], and Radiation Therapy and Cancer Center [R. S.], Howard University, Washington, DC 20060; and National Institute of Mental Health, NIH, Bethesda, MD 20892 [S. D. D.]

ABSTRACT

Esophageal cancer ranks among the 10 most common cancers worldwide and is almost invariably fatal. The detailed genetic repertoire involved in esophageal carcinogenesis has not been defined. We have shown previously that the esophageal squamous cell carcinoma genome exhibits a frequent loss of heterozygosity (LOH) in the pericentromeric region of chromosome 18. To construct a fine deletion map, we screened 76 new samples composed of microdissected esophageal squamous cell carcinoma and matched morphologically normal epithelial cells using closely spaced markers. Maximal LOH frequency (54%) was displayed by D18S542 on 18p11.2. The pattern of LOH in selected patients indicated that the short region of overlap extends 3 cM on either side of D18S542. On the long arm of chromosome 18, the highest frequency of allelic loss (42%) was detected by D18S978 on 18q12.2-q21.1. This analysis revealed a short region of overlap of ∼0.8 cM. These findings further implicate unreported tumor suppressor genes encoded by 18p11.2 and 18q12.2 in esophageal squamous cell carcinogenesis and they indicate a refinement of their map location.

INTRODUCTION

Squamous cell and adenocarcinoma comprise the two most common cell types of esophageal cancer (1). Squamous cell carcinoma is common in all populations examined, and it occurs more frequently in African-Americans than in Caucasians, whereas adenocarcinoma is more prevalent among Caucasians (2). Tobacco and alcohol consumption represents major environmental risk factors for this malignancy (3), but the molecular events leading to esophageal cancer and the genetic components that are mutated at the inception and course of the neoplasm are largely unknown. Unraveling the genetic factors could provide targets for the development of more effective therapeutic agents. A variety of tumor suppressor genes have been implicated in esophageal cancer (4, 5). Chromosomal abnormalities and LOH in which random isolated markers are used have been associated with esophageal tumors (6, 7). Analysis of chromosome 18 has focused mainly on 18q21, where two known tumor suppressor genes, DCC (8) and DPC4 (9) have been mapped. However, only infrequent deletions of DCC and DPC4 have been found (7, 10). Our previous effort to screen chromosome 18 using microsatellite markers that span the entire length of the chromosome detected a broad peak of allelic deletion spanning the p and q sides of the centromere (11). Within this peak, D18S542 on 18p and D18S978 on 18q displayed LOH frequencies of 38 and 72%, respectively. These findings suggest the involvement of tumor suppressor genes located on 18p and 18q in esophageal carcinogenesis. To obtain a global representation of the sites that are lost, gained, and amplified in ESC, we conducted CGH (12). CGH identified multiple chromosomal imbalances, including some on chromosome 18. Chromosomal losses detected in this study overlap with those previously reported in other squamous cell neoplasms (13, 14).

In the present study, we typed 11 new polymorphic markers which flanked D18S53 and D18S978. These markers including D18S53 and D18S978 were used to screen for LOH with the use of 76 different DNA templates derived from microdissected ESC cells. Here, we report additional evidence for allelic loss on 18p and 18q and a narrowing of these deleted regions reported in our previous study (11).

MATERIALS AND METHODS

Patients and Samples. The present study includes archival tissue from 76 patients with ESC from the Department of Pathology, Howard University. There were 57 male and 19 female patients; age range was from 40 to 88 years (mean, 62.8 years). There were 73 African-Americans and 3 Caucasians. Eleven tumors (14%) were located in the upper esophagus, 40 (53%) were in the middle esophagus, and 25 (33%) were in the lower esophagus. The American Joint Committee on Cancer

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2 Contributed equally to this work.
3 To whom requests for reprints should be addressed, at Medical Oncology Section, Department of Veterans Affairs Medical Center, 50 Irving Street NW, Washington, DC 20342. Phone: (202) 745-841; Fax: (202) 745-841; E-mail: WADLEY.G+_/@ WASHINGTON.VA.GOV.

4 The abbreviations used are: LOH, loss of heterozygosity; ESC, esophageal squamous cell carcinoma; CGH, comparative genomic hybridization; MMRF, Marshfield Medical Research Foundation; LDB, Genetic Location Database.
staging showed 7 stage I, 9 stage IIA, 16 stage IIB, 24 stage III, and 20 stage IV.

Microdissection and Genomic DNA Extraction. A total of 76 new cases of formalin-fixed, paraffin-embedded archival samples of ESC that had been reviewed by pathologists (R. J. R. and M. A. J.) were used to microdissect morphologically malignant and normal cells. One 5-μm section was cut and stained with H&E and used as a guide for microdissection. Areas containing tumor and normal cells were microdissected from six de-paraffinized 5-μm sections with a sterile 25-gauge needle under a microscope (Stereostar, A.O. Scientific). Microdissected cells were collected in an Eppendorf tube and resuspended in 30 μl of lysis buffer [10 mM Tris-HCl (pH 8), 25 mM EDTA (pH 8), and 0.5% SDS] containing 1 mg/ml proteinase K, incubated overnight at 55°C (15). DNA was isolated as described previously (16).

PCR-LOH Analysis. PCR amplification was conducted by using microsatellite markers that map to 18p (D18S542, D18S1158, D18S1150, D18S1116, D18S453) and to 18q (D18S67, D18S451, D18S978, D18S65, D18S455, D18S1094, D18S460). D18S1104 mapped to 18p11-18q12. These markers were purchased from Research Genetics (Huntsville, AL). Each PCR was performed in a 10-μl reaction mixture containing 1 μl of genomic DNA from microdissected cells, dNTP mix (1.25 mM dGTP, dATP, dTTP, dCTP), 10 mM Tris-HCl (pH 8.6), 1.5 mM MgCl₂, one 5’-32P-end-labeled primer, and 0.5 unit of AmpliTaq (Perkin-Elmer). PCR was performed with an initial denaturation at 94°C for 4 min followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The amplicons were denatured at 95°C for 5 min in 10 μl of sequence loading buffer, and electrophoresis was conducted on a 6% sequencing gel. Autoradiography was done either at room temperature or at ~70°C with an intensifying screen. LOH was defined as either the absence of one allele or a decrease in intensity of one allele by at least 50%. Gels were read by two reviewers (J. D. K. and R. G. W.) and checked by a third reviewer (S. D. D-W.) when reading differences occurred. Reading discrepancies were resolved by repeating the experiments.

RESULTS

To refine the deletion map of the regions flanking the centromere, we typed 76 new cases of archival squamous esophageal tumor tissues using closely spaced markers on 18p11.2 and 18q12-q21. LOH analysis was performed with DNA derived from microdissected tumor cells and corresponding morphologically normal epithelial cells from each individual case. The success rate of PCR amplification from these templates for LOH analysis was ~90%.

We used 13 highly informative genetic markers mapping to 18p11.2 and 18q12-q21.1, with heterozygosity ranging from 65% (D18S1094) to 83% (D18S453). In the samples studied here, the number of informative cases was highest with D18S978 (89%) and lowest with D18S1116 (57%).

On 18p11.2, we scanned a ~6-cM region using five microsatellite markers based on the genetic maps of the Center for Fig. 1 Allelic loss on chromosome 18 in esophageal cancer detected by D18S542 and D18S978. Lanes N and T, normal and tumor banding patterns, respectively.

Fig. 2 Chromosomal pattern of LOH in selected cases. ●, LOH; ○, no LOH; ○●, noninformative site. Genetic distances shown are based on the LDB (17).
Medical Research of the MMRF (www.marshmed.org/genetics/) and the LDB (cedar.genetitics.soton.ac.uk/public_html/) (17). D18S542 displayed the highest frequency of LOH (54%; Figs. 1–3). Note that D18S542 and D18S53 amplify the same locus (http://gdbwww.gdb.org/). The flanking marker D18S1116 displayed a 20% LOH frequency. D18S453, 2.25 cM centromeric of D18S542 by MMRF and 3.172 cM by LDB detected LOH in 8 of 65 informative cases (Fig. 3). The frequency of allelic deletions detected by markers telomeric of D18S1116 decreased considerably.

We searched for the shortest commonly deleted region from among the LOH patterns displayed by the 76 cases. In Fig. 2, Patient 50 exhibited LOH at both D18S542 and D18S1116, but not in markers flanking these loci. This patient did not show LOH anywhere else on the chromosome. In the MMRF map, D18S542 and D18S1116 are at the same genetic location whereas in the LDB map (17) the distance between these markers is ~1 cM. D18S1158 is 2.3 cM telomeric of D18S1116 and D18S453 is 2.3 cM centromeric of D18S542. Based on this analysis the shortest region of overlap extends ~3 cM on either side of D18S1116.

Seven microsatellite markers mapping to 18q12-q21 and spanning ~6 cM were used for LOH screening. In this region, D18S978 exhibited the highest frequency of LOH (42%; Fig. 3). This contrasts with our prior study where a smaller number of biopsies were used and the frequency was higher in D18S978 than in D18S542 (17). In the present study, the allelic deletions extended to ~0.9 cM telomeric of D18S978 (Figs. 1 and 2). The LOH frequencies are shown in Fig. 3. In 25% of the cases, allelic deletions occurred at both the 18p11.2 and 18q12-q21 regions (data not shown).

In this panel of samples, the shortest region of overlap on 18q12-q21 was smaller than found on 18p11.2. Cases 09 and 18 displayed patterns that permitted narrowing of the location of the potential tumor suppressor gene to ~0.8 cM (Fig. 2).

**DISCUSSION**

Recently, we found a broad region of LOH on chromosome 18 encompassing both sides of the centromere, from 18p11.2 to 18q12-q21.1 (11). In that study, typing was done on template DNA derived from ESC biopsies. Because LOH could be masked by the presence of normal cells, which could lead to false negatives, we decided to isolate ESC cells separately from morphologically normal epithelial cells by microdissection and to prepare DNA representing each genome. To permit a narrowing of the critical region, we examined a relatively large number of cases and increased the marker density by selecting markers that closely flank D18S53 and D18S978, markers that displayed the highest frequency of allelic loss in our prior study (11). There were 11 such new markers. Our findings indicate that LOH on 18p11.2 and 18q12-q21 is frequent in ESC cells as displayed by D18S542 and D18S978, respectively. However, lower LOH frequencies using the same markers in the present study compared with our previous study (11) probably indicate a more accurate representation of the actual LOH frequencies because of the increased sample size.

Prior studies have implicated 18p in other tumors. In esophageal adenocarcinoma, LOH was detected by D18S53 (18). Recently, Tran et al. (19) reported a novel region on 18p, D18S452, that was frequently deleted in tumors of the lung, brain, and breast. These, coupled with our present findings, increase the evidence for an unknown tumor suppressor gene on the short arm of chromosome 18. Two genes that might qualify as positional and functional candidates are ZFP161 (20), a c-myc repressor, and PTPRI (21), a tyrosine phosphatase receptor.

Our initial LOH study suggested the existence of another unreported tumor suppressor gene for ESC on 18q12-q21 (11). In squamous cell carcinoma of the head and neck, D18S39, a marker located on 18q12 detected a high percentage of LOH...
Similarly, loss of 18q is reported to be a prognostic factor for poor patient survival with head and neck cancer (23) or with cohesive-type gastric cancer (24). Also, Frank et al. (25) reported loss of 18q in metastatic and locally recurrent squamous cell tumors, but not in primary tumors from the same patients. Data from the present study on a larger number of samples suggest that the critical region of allelic loss on 18q is proximal to DCC.

The presence of a fragile site at 18q12.2 (FRA18A) may predispose the chromosome to breakage and rearrangement (26). For example, chromosomal breakpoints in FRA3B which is in the FHIT gene on chromosome 3p14.2 has been implicated in both Barrett’s metaplasia and esophageal adenocarcinoma (27), pancreatic cancer (28), and renal cell cancer (29). Thus far, FRA18A has not yet been reported to be involved in any type of cancer. Folate concentration and expression of fragile sites have an inverse relation (30). An increase in fragile sites has been demonstrated in older people and smokers (31). The archival samples in our present study were obtained from patients, the majority of whom were smokers.

The 18q12.2 region also encodes four potential candidate genes. Desmogleins (DSG1 and DSG3), which are expressed in simple epithelium of the colon and squamous stratified epithelium (32), have been reported to have reduced expression in squamous cell type carcinomas (33, 34). ZNF24 (35) and N-acetylgalactosaminyltransferase T1 (36) display reduced or no expression in squamous cell type carcinomas (37). There is growing evidence that squamous cell type carcinomas share a locus on chromosome 18q12 and that this deletion may be a late event associated with tumor progression (12).

In >25% of the cases studied here, deletions on 18p were concomitant with those on 18q. Our previous CGH analysis of ESC samples detected a high frequency loss of 18q (76%), gain of 18p (30%), and a concurrent loss (30%) of 18q and 18p (12). Losses of chromosomal regions smaller than 10–15 Mb are beyond the detection limit of CGH but would be detectable through PCR-LOH analysis (38). Our findings of frequent codeletion of 18p and 18q indicate that selective pressures for the two events are likely to be different and therefore additive.

Our study suggests the existence of two potential tumor suppressor genes for ESC located on 18p11.2 and 18q12.2. Moreover, the use of closely spaced markers within the LOH regions on microdissected cells permitted the refinement of the region that should facilitate the cloning of these tumor suppressor genes.

REFERENCES


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