Advances in Brief

Frequent Loss of Heterozygosity in the Region Including BRCA1 on Chromosome 17q in Squamous Cell Carcinomas of the Esophagus

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Abstract

Ninety-four esophageal squamous cell carcinomas were examined for loss of heterozygosity at several loci on the long arm of chromosome 17 (17q), using restriction fragment length polymorphism markers. Loss of heterozygosity was observed in 56 (62%) of 91 tumors that were informative with at least one marker. Comparison of these results with clinico-pathological data indicated that the losses on chromosome 17q had occurred at an early stage of carcinogenesis. Detailed deletion mapping in these tumors revealed that the region commonly deleted was within the segment between loci defined by two markers at chromosomal band 17q21.3.

Introduction

Growing evidence suggests that accumulation of multiple alteration in protooncogenes and tumor suppressor genes is responsible for and crucial to the genesis and/or progression of tumors (1–3). Because inactivation of a gene is often brought about by loss of the chromosomal segment where it resides, frequent observations of allelic losses among genetic markers at a specific chromosomal locus in a tumor imply the presence there of a putative tumor suppressor gene.

In esophageal cancers, multiple chromosomal deletions (5q, 13q, 17p) (4–7), mutation of the p53 gene (8, 9), and amplification of the chromosome 11q13 region (10) have been reported; mutations in each of the tumor suppressor genes and oncogenes implicated by those studies as well as unknown loci may contribute to transformation of a normal cell to a malignant cell and/or to progression (including grade of malignancy and metastasis). In our previous study, we examined LOH for human esophageal squamous cell carcinomas at a large number of loci on all autosomal chromosomes and detected frequent allelic losses on the long arm of chromosome 17 (17q). Interestingly, LOH on this chromosomal arm was significantly higher in tumors of female patients than in those in male patients.

To define this region further, we screened 94 ESCs with 15 RFLP markers on 17q and constructed a detailed deletion map. In addition, we compared the LOH results with clinico-pathological data to determine when during ESC tumorigenesis this genetic alteration occurs.

Materials and Methods

Samples. Tumors and normal corresponding tissues were obtained at surgery from each of 94 patients with esophageal squamous cell carcinomas. All tissues were dissected in the operating room, frozen immediately, and stored at −80°C until isolation of DNA. Pathological classification was based on criteria in Ref. 11.

DNA Extraction from Tissues and Southern Blotting. Extraction of DNA from tumors and from the corresponding normal tissues was carried out according to methods described elsewhere (12). For Southern blot hybridization 5 μg of each genomic DNA sample were digested with an appropriate restriction enzyme; electrophoresed in a 0.8%, 1.0%, or 1.2% agarose gel; transferred to nylon membrane in 0.1 N NaOH-0.1 M NaCl; and subjected to UV cross-linking.

Probes and Hybridization. The 15 cosmid markers used in this study are listed in Table 1. All cosmid markers were reported previously; each had been physically localized on chromosome 17q by fluorescence in situ hybridization (13). TaqI polymorphisms of CI17-24 and CI17-477, an MspI polymorphism of CI17-28, EcoRI polymorphisms of CI17-592 and CI17-675, and TaqI and MspI polymorphisms of CI17-835 were recently detected; RFLP of other cosmid markers was previously reported (13). A polymorphic restriction fragment of each cosmid was purified by agarose gel electrophoresis and used as a hybridization probe to examine LOH. Probes were labeled with 32P-dCTP by random primer extension (14). Prehybridization, hybridization, and autoradiography were carried out as described elsewhere (12). The membranes were stripped in 0.4 N NaOH and repeatedly hybridized.

Determination of Allelic Dosage. To ascertain loss of hybridization signal, the intensity of each polymorphic allele was quantified by densitometry (GS-300 scanning densitometer; Hoefer Scientific Instruments). The signal intensities of alleles from tumor tissue were compared with those of corresponding normal alleles. An allele was considered to be lost when signal was reduced by more than 50%.

Results and Discussion

The frequencies of LOH at each of 15 RFLP loci are listed in Table 1. Ninety-one tumors were informative for at least one locus, and 56 of them (62%) showed LOH for at least one locus on chromosome 17q. Two types of chromosomal loss in tumors were noted; the first, involving loss of an entire long arm (probably due to mitotic nondis-
DELETION ON 17q IN ESC

Fig. 1. (a) Autoradiograms from selected Southern blots showing partial or interstitial deletions on chromosome 17q; tumor 23 had lost one allele at the CI17-701, CI17-477, and CI17-28 although it retained both alleles at a more distal locus (CI17-675); tumor 106 lost one allele at the CI17-477 locus but retained heterozygosity at the CI17-701 locus. The left lane of each pair contains DNA from the patient's normal tissue (N), the right lane contains DNA from the tumor (T). Probes are indicated below each pair of lanes; C, constant band; arrows, polymorphic bands. (b) Schematic representation of partial and interstitial deletions on chromosome 17q in ESCs. Top abscissa, case numbers; ordinate, probe names. •, LOH; O, retention of both alleles; vertical bar on right, region commonly deleted. The location of mfd188 (D17S579), THR1A, and RARA were described previously (17, 20).

junction), was observed in 36 tumors (64%); the other was partial or interstitial deletion of 17q, which was observed in 20 tumors (36%). Fig. 1a shows Southern blot analyses of two cases that revealed partial or interstitial deletions on chromosome 17q in ESCs. Top abscissa, case numbers; ordinate, probe names. O, LOH; C, retention of both alleles; vertical bar on right, region commonly deleted. The location of mfd188 (D17S579), THR1A, and RARA were described previously (17, 20).

of tumor invasion was observed. 17q deletion was detected even in tumors at an early stage (Table 2), where no invasions to muscular layer or lymph node metastases were evident. Hence, inactivation of this putative tumor suppressor gene probably is an early event in esophageal carcinogenesis.

Fine-scale molecular mapping revealed that the region commonly deleted among primary ESCs overlapped the region where other studies have indicated the presence of a tumor suppressor gene (BRCA1) associated with hereditary and sporadic forms of breast and ovarian cancers (15–17). Further, we have previously reported that the frequency of LOH on 17q was significantly higher in tumors of female patients. The results of LOH studies on 17q and all autosomal chromosomes in 19 female ESC cases are summarized in Table 3. This result also implies that deletion on 17q is predominant to other chromosomal deletions in female cases.

Our results imply that a tumor suppressor gene for esophageal

Table 2 Correlation of LOH on chromosome 17q with pathological data: lymph node metastasis (A); depth of tumor invasion (B); and pTNM classification (C)

<table>
<thead>
<tr>
<th>A. Pathological lymph node metastasis</th>
<th>n(-)</th>
<th>n(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOH on 17q</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>+</td>
<td>31</td>
<td>25</td>
</tr>
</tbody>
</table>

B. Pathological depth of tumor invasion

<table>
<thead>
<tr>
<th>pT1</th>
<th>pT2</th>
<th>pT3</th>
<th>pT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases examined</td>
<td>4</td>
<td>12</td>
<td>75</td>
</tr>
<tr>
<td>LOH(-)</td>
<td>1</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>LOH(+)</td>
<td>3</td>
<td>8</td>
<td>43</td>
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</table>

C. pTNM stage

<table>
<thead>
<tr>
<th>No. of cases tested</th>
<th>I</th>
<th>IIA</th>
<th>IIB</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allelic losses/informative cases (%)</td>
<td>(100)</td>
<td>(68)</td>
<td>(50)</td>
<td>(61)</td>
<td>(44)</td>
</tr>
</tbody>
</table>

*pTNM, pathological tumor-nodes-metastasis classification.

Table 3 LOH on 17q and additional allelic losses in tumors of female patients

- LOH on 17q was observed in 16 of 19 female cases with at least one of 15 RFLP markers examined.

- Probes used in this study were: MCTX58(lp), HHH106(lq), TBAB5.7(2p), YNH24(2q), CI3-515(3p), CI3-373(3p), EFD64.2(3q), YNZ32(4p), EFD139.1(4q), APC(5q), L5.71(5q), CI6-7(6p), CI6-111(6q), RM17-4(7p), CI2Z-27(7q), CI2-215(8p), CI8-134(8q), HHH28(9p), MCT112(9q), EKZ19.3(9q), MHZ15(10p), EFD57.1(10q), c-Ha-RAS(11p), MCMP1(11q), THHH14(12p), YNH15(13q), CM1011(14q), MHH55(15q), MHH565(16p), CI52-209M1(16q), YNH22(17p), B74(18p), OS41(18q), JcZ3.19(19p), EFD4.2(19q), MCM620(20q), MCT15(21a), EFZ31(22a).

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squamous cell carcinoma may be located very close to the putative BRCA1 gene or that inactivation of BRCA1 itself may play an important role in the genesis or development of esophageal squamous cell carcinoma. It is still unclear which of these possibilities is more likely; however, in some families prone to ovarian cancer, siblings are affected with cancers of other anatomic sites more often than would be expected by chance (18, 19). Further investigations will be required before its role can be clarified.

Acknowledgments

We are grateful to Kiyoshi Noguchi (Cancer Institute, Tokyo, Japan) for technical assistance and to Dr. Hiroshi Sugawara (Tohoku University, Sendai, Japan); Drs. Mamoru Ueda, Mitsuru Emi, and Hiroko Saito (Cancer Institute, Tokyo); Drs. Wataru Adachi and Hitoshi Yokoyama (Shinshu University, Nagano, Japan); and Dr. Ping Yu-Min (Hebei Cancer Center, Hebei, China) for helpful advice.

References

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