Frequent Loss of Heterozygosity on Chromosome 9 in Adenocarcinoma and Squamous Cell Carcinoma of the Esophagus

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Materials and Methods

Tissue Preparation and DNA Extraction. Sixty tissue samples were obtained at endoscopy or surgery from patients with squamous cell carcinoma or adenocarcinoma of the esophagus. All specimens were frozen in liquid nitrogen immediately after removal. Homologous normal tissue was obtained from normal gastric mucosa or blood in all patients. DNA was extracted as described previously (37). Seventeen of these samples were adenoscarcinomas, while 43 were squamous cell carcinomas.

LOH Assays. PCR was used to amplify portions of the chromosomal region between 9p13 and 9p22. Oligonucleotide primers were synthesized for the following 6 anonymous DNA markers, with chromosomal localizations given in parentheses: D9S162 (9p22—p23), D9S163 (9p21—q21), D9S126 (9p21—q21), D9S104 (9p21—q21), D9S171 (9p21—q21), and D9S165 (9p21—q21); and the IFNA locus (9p22). Multiplex PCRs were performed (38). Between 10 and 50 ng of genomic DNA were amplified in a 10-μl reaction containing 0.25 unit of Taq polymerase in 10 mM Tris-HCl, pH 8.6, with 1.5 mM MgCl2, 0.2 mM concentrations of each deoxynucleoside triphosphate, and 0.2 μCi of [32P]dCTP. PCR products were denatured in 95% formamide, electrophoresed on 6% denaturing polyacrylamide gels, and visualized by autoradiography.

LOH was defined as visible absence or a >50% reduction in the signal of one allele in comparison with the other allele. Eight of 17 patients (47%) with adenocarcinoma manifested LOH, while 28 of 43 (65%) with squamous cell carcinoma showed LOH. LOH was most frequent at loci D9S171 (19 of 23, or 83%) and D9S165 (24 of 32, or 75%). These data support the hypothesis that a tumor suppressor gene or genes located on this portion of chromosome 9p exerts an effect on esophageal cancer development.

Results

Normal and tumor DNAs from 60 patients were screened for LOH on chromosome 9p with primers from the following seven loci: D9S162, IFNA, D9S171, D9S126, D9S104, D9S165, and D9S163. Thirty-six of 60 tumors (60%) exhibited LOH at one or more loci. Eight of 17 patients (47%) with adenocarcinoma manifested LOH, while 28 of 43 (65%) with squamous cell carcinoma showed LOH. LOH was most frequent at loci D9S171 (19 of 23, or 83%) and D9S165 (24 of 32, or 75%). These data support the hypothesis that a tumor suppressor gene or genes located on this portion of chromosome 9p exerts an effect on esophageal cancer development.

Discussion

The above results suggest that a locus or loci on chromosome 9p is or are involved in the genesis or progression of esophageal carcinomas, including both adenoscarcinomas and squamous cell cancers. Homozygous or heterozygous deletion of this region has been shown to occur frequently in a wide variety of tumor types (14–36). The target of these deletions is not yet known; one potential tumor sup-
pressor gene in this region, MTS1, is mutated only rarely in the vast majority of primary tumors showing 9p LOH, although it is mutated commonly in cell lines derived from tumors (39–41). The major exception to this rule seems to be esophageal squamous cancer, in which it has recently been shown that the majority of primary tumors showing 9p LOH, although it is mutated commonly in cell lines derived from tumors (39–41). The location of MTS1 on chromosome 9p is (are) important in this and other cancers. Alternatively, this finding may mean that MTS1 can be inactivated by mechanisms other than mutation, such as homozgyous deletion of the entire gene; or that MTS1 inactivation is codominant; i.e., loss of one copy confers a cancer-promoting effect.

In our study, LOH occurred most frequently at locus D9S171 (occurring in 19 of 23, or 83%) and D9S165 (24 of 32, or 75%), while it was least common at D9S104 (8 of 34, or 24%) and D9S126 (9 of 22, or 41%). These discrepancies may suggest that a tumor suppressor gene or genes is or are located closer to D9S171 and D9S165 than to D9S104 and D9S126. The location of MTS1 relative to these markers is between IFNA and D9S171; thus, the high frequency of LOH at D9S171 is consistent with a role for MTS1 (or a gene located very close to it) as the target of LOH. However, mutational data do not completely support this role for MTS1, as discussed above. In agreement with our findings, studies of other tumor types have also shown a preponderance of deletions near the D9S171 (20, 22, 32) and IFNA (15, 16, 20, 22, 32) loci; two of these studies also supported the existence of an additional tumor suppressor gene on the long arm of chromosome 9 (16, 22).

In this series, LOH of chromosome 9p was slightly more common in squamous cell carcinomas (56%) than in adenocarcinomas (47%); however, this difference did not achieve statistical significance. Esophageal adenocarcinomas and squamous cell carcinomas are similar in the prevalence of p53 mutations (43–46) and ras mutations (12, 13). Nevertheless, a precedent for molecular differences between esophageal adenocarcinoma and squamous cell carcinoma exists in the frequency of microsatellite instability (9), which is rare in the latter but common in the former type of cancer.

In summary, the current findings add esophageal squamous cell carcinoma and adenocarcinoma to the growing list of cancers in which a gene or genes on chromosome 9p is or are involved in tumorigenesis or tumor progression, thereby strengthening the importance of this chromosomal region in human cancer.
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References


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