Frequent Loss of Chromosome 9p21–22 Early in Head and Neck Cancer Progression

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Abstract

In order to define more clearly the role of chromosome 9 loss in head and neck squamous cell carcinoma (HNSCC), 29 invasive carcinomas and 17 preinvasive lesions were analyzed for loss of heterozygosity (LOH) on chromosome 9. We found LOH in 21 of 29 (72%) HNSCC tumors using highly polymorphic microsatellite markers. In 17 of 21, LOH was found at all informative sites on the p arm with no LOH of the q arm. Further mapping in tumors, with partial LOH of the 9p arm, localized a common region of loss between markers D9S165 and D9S156. Deletion of this region on chromosome 9 has been found in several other tumor types implying the presence of a tumor suppressor gene at this locus. The inactivation of a tumor suppressor gene on chromosome 9p may represent the most commonly described genetic alteration in HNSCC. A similar incidence of allelic loss on chromosome 9p was identified in 12 of 17 (71%) preinvasive lesions. The identical frequency of loss in preinvasive and invasive lesions suggests that loss of 9p is an early event in HNSCC progression.

Introduction

Malignant transformation in tumor progression results from a series of genetic changes (1). Determining the nature and timing of these changes will aid in understanding the biology of each tumor type and could facilitate the development of new diagnostic and therapeutic strategies.

The inactivation of tumor suppressor genes is among the most common molecular events that contribute to tumorigenesis. Although a variety of mechanisms exist for tumor suppressor gene inactivation, loss of chromosomal segments in tumor cells has proven useful in mapping regions containing putative tumor suppressor genes (2, 3). The relative importance of these genes to HNSCC progression remains unclear. Cytogenetic studies have found no consistent chromosomal changes, and results apparently vary with tissue culture conditions (4). Immunohistochemical screening for p53 showed intense staining in HNSCC tumors and cell lines and led to the identification of p53 gene mutations in HNSCC (5–7). Analysis of over 100 lesions revealed that the incidence of p53 alterations in preinvasive lesions was 19% and increased to 43% in invasive HNSCC (8).

To identify additional tumor suppressor gene loci, we screened HNSCC tumors for LOH, using markers on each autosomal arm (9). The highest frequency of allelic loss was found on chromosome 9p and was present in 72% of infiltrative tumors. In this study, a panel of highly informative microsatellite markers was used to identify and further characterize LOH for portions of chromosome 9 in both invasive and preinvasive lesions. Our results identify a distinct region of loss on chromosome 9p, which has previously been implicated in the progression of other neoplasms. Furthermore, loss of 9p occurs with the same frequency in preinvasive and invasive lesions suggesting loss of this putative tumor suppressor locus early in the progression of HNSCC.

Materials and Methods

Invasive HNSCCs obtained fresh from surgical resection were processed as described previously (8). Clinical characteristics of the patients and stage at the time of surgical resection are listed in Table 1. Tumor sections with greater then 60% tumor tissue were used to isolate DNA for further analysis.

Paraffin blocks of noninvasive lesions (4 severe dysplasias and 13 carcinomas in situ) were obtained and a hematoxylin/eosin-stained, formalin-fixed biopsy section was viewed under the microscope for each lesion. The corresponding neoplastic tissue from unstained 4-μm paraffin sections was microdissected away from nonneoplastic cells. The tumor tissue was deparaffinized in xylene, digested with sodium dodecyl sulfate/protease K and DNA was extracted with phenol/chloroform followed by ethanol precipitation (10).

Oligonucleotide primers for microsatellite PCR analysis were obtained from Research Genetics (Huntsville, AL) and listed in Fig. 2. One of the primers was labeled with [γ-32P]ATP using T4-polynucleotide kinase (New England Biolabs). Fifty ng of genomic DNA were subjected to 30 cycles of PCR amplification as described previously (10). For amplification of D9S165, Taq polymerase (Boehringer Mannheim) was added only after preheating samples to 95°C for 3 min. PCR products were separated by electrophoresis in denaturing 8% urea-polyacrylamide-formamide gels (11) followed by autoradiography. For informative cases allelic loss was scored if the intensity of one allele was at least 50% reduced in the tumor DNA as compared with the normal.

Results

We selected well spaced markers from the highly informative microsatellite loci that have been identified on chromosome 9 (12). Through PCR amplification of microsatellite markers, 29 invasive HNSCCs were examined for allelic loss of chromosome 9 and 72%
LOSS OF CHROMOSOME 9p21–22 IN HEAD AND NECK CANCER

Fig. 1. Loss of heterozygosity analysis for two selected cases (H1, H2) of invasive HNSCC. Informative microsatellite markers were amplified from DNA derived from normal blood (left lane) or from tumor (right lane). Case H1 shows partial loss of 9p markers including D9S200, D9S161, D9S171, IFNA, and D9S162. Case H2 shows loss on 9p distal (telomeric) from D9S165. Relative position of markers on physical map is shown in Fig. 2.

(21 of 29) of invasive HNSCs showed allelic loss of at least five markers on the p arm. Fourteen % (4 of 29) revealed allelic loss for all the markers analyzed on the chromosome. No partial allelic losses confined to the q arm were identified in these tumors.

Fine mapping is necessary for localization of putative tumor suppressor genes (2, 3). We further analyzed chromosome 9 with a total of 15 microsatellite markers. Several tumors showed partial LOH on 9p as illustrated in Fig. 1. Fig. 2 shows the distribution of allelic losses in 5 representative tumors of the 29 cases analyzed. The minimal area of allelic loss was confined to 9p21–22, defining a common region of loss between the markers D9S156 and D9S165.

To determine the relative timing of chromosome 9p alterations in HNSCC progression, we analyzed allelic loss of chromosome 9p in preinvasive lesions. Four markers (D9S144, D9S156, D9S162, and IFNA) on chromosome 9p that spanned the common region of loss identified in invasive lesions were used. Our analysis revealed LOH in 12 of 17 (71%) informative preinvasive tumors (Fig. 3), essentially the same frequency as in invasive lesions. Despite the presence of inflammatory cells, preinvasive lesions could still be readily scored for allelic loss. There was no apparent difference in 9p loss between severe dysplasia (3 of 4) or carcinoma in situ (9 of 13).

Discussion

The progression of tumors from early noninvasive adenomatous lesions to invasive primary tumors has been well documented for colon cancer. Specific genetic changes have been correlated with histopathological lesions involved in neoplastic progression (1, 13). Although p53 mutations have been found in a variety of tumors (14) including head and neck cancers (8), few specific genetic alterations have been described in HNSCC primary tumors. We discovered that loss of chromosome 9p appears to be involved in the majority (72%)
p53 mutations occur in approximately 20% of preinvasive lesions and characteristics. These three cancer types are often linked to tobacco use and have a distinct propensity for multicentric presentation. Iden
bladder, and lung cancer all share certain epidemiological and clinical findings of 9p loss in preinvasive HNSCC. Although the relative the progression of bladder cancer (21, 22) in concordance with our
tivation of p53 as the most common genetic change in human tumors. A putative tumor suppressor gene at this locus may well rival the inac
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