Two Distinct Regions of Loss on Chromosome Arm 4q in Primary Head and Neck Squamous Cell Carcinoma

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Objective: To more clearly define the frequency and the regions of chromosome arm 4q loss in head and neck squamous cell carcinoma.

Design: A retrospective microsatellite analysis of DNA from previously microdissected primary tumor samples.

Setting: Academic medical center.

Patients and Methods: One hundred primary tumor samples from patients with head and neck squamous cell carcinoma were analyzed for loss of heterozygosity on the long arm of chromosome 4. The Kaplan-Meier method was used to estimate survival for 97 patients for whom clinical data were available. The Cox proportional hazards model was used to compare survival, and logistic regression was used to search for associations between clinical tumor characteristics and 4q status.

Results: Analysis of 33 polymorphic microsatellite markers identified 51 samples (51%) exhibiting loss of heterozygosity of 4q in at least 1 locus. Eighteen tumors revealed loss at all informative markers, indicating monosomy or complete deletion of 4q. Thirty-three tumors displayed partial loss of heterozygosity and delineated 2 minimal areas of loss at 4q23 and 4q28. Eleven tumors displayed loss solely at the 4q23 region, 13 tumors displayed deletions confined to the 4q28 region, and 9 tumors displayed selective loss at both regions. A separate analysis in a subset of 94 primary head and neck tumors was done to further delineate the minimal area of chromosomal loss at 4q23. Analysis of 8 markers in this region allowed us to identify the smallest region of loss between markers D4S2986 and D4S1564 (a distance of 2 centimorgans). Review of the clinical records of 97 patients revealed no statistically significant association between 4q status and any clinical variable, including survival.

Conclusion: These results confirm a high frequency of chromosome arm 4q loss in primary head and neck squamous cell carcinoma and might demarcate 2 novel putative suppressor loci involved in progression of this carcinoma.

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fied a minimal area of loss at 4q2324 and a separate region at 4q2829.

RESULTS

We selected 33 well-spaced markers from informative microsatellite loci previously identified on chromosome arm 4q.10 We then amplified these microsatellite markers by PCR from primary HNSCC tumor and matched control DNA to screen for allelic loss. Fifty-one (51%) of the 100 tumors displayed loss of at least 1 marker on 4q. Eighteen of these samples demonstrated loss at all informative markers, indicating the probable presence of monosomy. The 33 remaining samples displayed only partial loss and helped define the 2 minimal regions of loss on 4q. We further analyzed the proximal region with 8 additional markers in a subset of 94 tumors to define the smallest region of loss.

Figure 1 denotes representative tumors defining the minimal areas of deletion on 4q. A small area of loss confined to 4q2324, between markers D4S2986 and D4S1364, is seen in tumor sample 5. Another small region of loss confined to 4q2829, between markers D4S247 and D4S2998, is seen in tumor sample 1072. A scarcity of markers in this region did not allow further mapping. These regions span physical distances of 2 and 11 centimorgans (cM), respectively. Figure 2 shows the pattern of chromosome arm 4q loss for 7 of 9 HNSCC tumors in which both regions were lost. These samples add evidence for the presence of 2 distinct regions of loss and potentially 2 novel tumor suppressor loci.

The Table provides data on the clinical characteristics of the patient population. The status of chromosome 4q was not found to correlate with any clinical variable, including patient sex, age at presentation, tumor stage, site, or the status of the cervical lymph nodes. Advanced age at presentation, the presence of cervical nodal metastases, and advanced tumor stage were each weakly associated with decreased overall survival. However, the status of chromosome arm 4q in the tumors had no impact on disease-free or overall survival. Chromosome arm 4q LOH conferred a hazard ratio of 1.3 for death by any cause, but \( P = .46 \) for the Cox proportional hazards survival analysis.

COMMENT

It has been established that cancer arises from a series of genetic changes. These alterations in DNA potentially lead to clonal outgrowth of cells, all of which will have a growth advantage initially provided by the parent cell.11 Discerning the timing and the nature of these alterations in HNSCC is crucial to biological and clinical comprehension of disease. Through analysis of the relative rate of molecular alterations in premalignant and invasive tu-
mors, it seems that deletion of chromosome arm 4q occurs late in HNSCC tumor progression.  

Initial allelotyping of HNSCC revealed LOH of 4q in many primary tumors tested at one microsatellite locus. We confirmed that 4q loss is a frequent genetic event in HNSCC, and our mapping with 33 dinucleotide markers reveals 2 minimal areas of loss on 4q. Although chromosome arm 4q loss has been described previously in other major tumor types, high-density mapping in primary HNSCC has allowed us to delineate 2 distinct loci at 4q23-24 and 4q28-29. These observations are consistent with the finding of more than 1 suppressor locus in many cases in which monosomy is present.  

Recently, investigators performed an allelotype of chromosome arm 4q, finding one large region of loss spanning 7 cM on 4q. Our study served to further extend their findings by defining a smaller minimal region of loss in the same arm (2 cM) and identifying another distinct area of loss on 4q. One of the 2 minimal regions defined on chromosome arm 4q in our study (4q23-24) has also been implicated in the progression of Hodgkin disease and cervical cancer. Polascik et al recently defined loss at 2 regions in chromosome 4, one at 4p and another at 4q22-23, in a large number of primary bladder cancers. It is of particular interest that chromosome arm 4q is now implicated in 2 types of squamous cell carcinoma (cervical and head and neck cancer). Our findings in head and neck and bladder cancers strengthen the possibility that a putative tumor suppressor gene located at 4q22-23 plays a role in the progression of multiple forms of cancer.  

It is now well established that tumor suppressor genes are a critical part of cancer progression. For example, it is known that TP53 and CDKN2A are 2 major tumor suppressor genes involved in HNSCC tumor progression. The study of these tumor suppressor genes has had a direct impact on patient treatment by augmenting diagnostic and prognostic tools. For patients with
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