Increased Polysomies of Chromosomes 7 and 17 during Head and Neck Multistage Tumorigenesis

Narin Voravud, Dong M. Shin, Jae Y. Ro, Jin S. Lee, Waun Ki Hong,2 and Walter N. Hittelman3

Departments of Medical Oncology [N. V. D. M. S., J. S. L., W. K. H., W. N. H.] and Pathology [J. V. R. J.]. The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

Head and neck cancer development has been proposed to represent a multistage process characterized by dysregulation of proliferation and differentiation and driven by an accumulation of genetic alterations in an anatomic field repeatedly exposed to carcinogens. To visualize the accumulation of genetic alterations during head and neck tumorigenesis and to determine the extent of the genetically altered field, we probed 25 squamous cell carcinomas of the head and neck and their adjacent premalignant lesions for numerical chromosome aberrations by nonisotopic, in situ hybridization using chromosome-specific centromeric DNA probes for chromosomes 7 and 17. Normal control oral epithelium from individuals free of cancer showed no chromosome polymy (i.e., cells with two chromosome copies), whereas histologically normal epithelium adjacent to the tumors showed numerous cells with polysomies for chromosomes 7 and 17. Moreover, the frequency of cells with polysomy increased as the tissues passed from histologically normal epithelium to hyperplasia to dysplasia to carcinoma. The finding of genotypic abnormalities in histologically normal and precancerous regions adjacent to the tumor supports the concept of field cancerization. The finding of progressive genetic changes as the tumor develops supports the concept of multistep carcinogenesis in the head and neck region. Such genotypic parameters could serve as biomarkers in the assessment of the risk of progression to malignancy and as intermediate end points in chemoprevention trials.

INTRODUCTION

Upper aerodigestive tract cancer is a significant public health problem throughout the world (1–3). Despite two decades of advances in surgery, radiotherapy, and chemotherapy, the long-term survival of affected individuals has only marginally improved (4). One approach to overcoming this problem is to prevent the onset of the disease by reducing exposure to suspected carcinogens, altering nutritional status, and treating high-risk individuals with chemopreventive agents. For example, recent clinical trials have indicated that 13 cis-retinoic acid can reverse or inhibit oral premalignant lesions (5) and prevent second primary tumors in patients with head and neck cancer (6).

The rational design of novel chemopreventive strategies requires an understanding of the fundamental events of tumor development. Chemoprevention trials would also benefit greatly by the elucidation of markers with which to identify individuals at highest risk and to monitor the efficacy of the chemopreventive agents in reversing or inhibiting tumorigenesis (7). Recent studies in several human tumor systems have suggested that tumorigenesis is a multistep process, driven by an accumulation of genetic alterations, resulting in dysregulation of proliferation and differentiation and in cell loss (8). Thus, potential biomarkers might include indicators of the degree of generalized and specific genetic change as well as the degree of cellular dysregulation in the tissue at risk for tumor development (9).

Head and neck cancer provides a unique model system for the study of tumorigenesis and the development of biomarkers for several reasons. First, head and neck cancer has been proposed to represent a field cancerization process since the whole aerodigestive tract epithelium is repeatedly exposed to carcinogenic insult (e.g., tobacco, alcohol), placing the entire epithelium at risk for tumor development (10, 11). The clinical correlate to the field hypothesis is a high frequency of multiple primary neoplasms in the aerodigestive tract and an increased risk of synchronous and metachronous second primary tumors (12, 13). Second, head and neck cancer is thought to represent a multistep tumorigenesis process whereby a series of events must occur prior to tumor development (14). This is evidenced by the presence of premalignant lesions adjacent to the tumor (15). While these clinical and histological findings support the notions of field cancerization and multistep tumorigenesis in the head and neck region, cellular biomarkers for these processes are still lacking.

One potential marker for the tumorigenesis process is the degree of genetic change in the tissue at risk. While a variety of cytogenetic changes have been described for head and neck tumors (16–18), a comprehensive list of specific genetic changes has been limited by impediments common to solid tumor cytogenetic studies, i.e., the low frequency of mitotic figures from direct preparations, suboptimal chromosome preparations, and significant complexity of cytogenetic changes (19). Identification of karyotypic changes in premalignant lesions is technically even more difficult with conventional cytogenetic procedures and has seldom been reported (20–22). Moreover, the spatial cellular distribution of genetic changes in premalignant and malignant lesions cannot be defined by conventional cytogenetic techniques because single cell preparations are required.

Recently, ISH techniques have been developed and allow the detection of chromosomal abnormalities directly in interphase cells (23–27). This method has now been applied to many types of solid tumors using tumor cell lines or dissociated tumor material (28–31). More recently, ISH has been adapted for use on formalin-fixed, paraffin-embedded tissue sections using nonisotopic, chromosome-specific DNA probes and enzyme-mediated (e.g., peroxidase) immunohistochemical procedures (32–36). This technique now allows direct visualization of chromosome changes in normal, premalignant, and tumor tissues without loss of tissue architecture.

In this article we report the use of ISH to better understand field cancerization and the multistep tumorigenesis process in human head and neck cancers. Formalin-fixed, paraffin-embedded tissue sections of head and neck squamous cell carcinomas that also contained adjacent normal and premalignant epithelium were subjected to ISH to visualize genetic alterations that accompany the transition from histologically normal epithelium through premalignant lesions to malignant lesions. To determine whether the detected genetic changes were associated with alterations in DNA content, adjacent tissue sections were also analyzed for DNA content using Feulgen staining and quantitation by image analysis. It was of particular importance for us...
to determine, first, how far back into the normal-to-premalignant transition numerical chromosome changes could be detected and second, whether the transition from premalignant to malignant lesions was accompanied by an accumulation of genetic changes. The present study demonstrates that genetic alterations can be detected in histologically normal epithelium and premalignant lesions adjacent to head and neck carcinomas and that an increase in the fraction of cells exhibiting numerical abnormalities accompanies the multistep tumorigenesis process.

MATERIALS AND METHODS

Selection of Tumor Specimens. Formalin fixed, paraffin-embedded tumor specimens were obtained from patients with head and neck cancer who were surgically resected at the University of Texas M. D. Anderson Cancer Center between 1990 and 1991. Twenty-five specimens containing both carcinomas and adjacent normal and premalignant lesions were selected for this study. Biopsy specimens of oral epithelium obtained from five normal individuals (i.e., cancer-free, nonsmoking volunteers) were used as normal controls. All specimens and hematoxylin-eosin-stained histological slides were reviewed by one pathologist to identify normal, hyperplastic, dysplastic, and tumor areas. Sections 6 μm thick were mounted on aminolalkylsilane-coated slides (Histology Control Systems, Glen Head, NY). The slides were stored at room temperature.

Prehybridization Procedure. Prior to hybridization, the tissue sections were subjected to five freeze-thaw cycles (i.e., placed at −70°C for 30 min followed by room temperature for 30 min, five times). The specimens were then incubated at 65°C overnight. Subsequently, the sections were dehydrated by immersing three times in xylene for 10 min each, followed by treatment with 100% ethanol, three times for 10 min each. From 100 to 150 μl of 0.4% pepsin (Sigma, St. Louis, MO) in 0.2 N HCl was applied to the slides, and coverslips were applied. The slides were placed at 4°C for 15 min to allow the pepsin to diffuse evenly throughout the tissue sections and then incubated at 37°C for 45–60 min to allow protein digestion. Preliminary experiments indicated that these steps helped to equalize hybridization efficiencies in different cell types within the tissue sections. After three washes with deionized water for 3 min each followed by dehydration through 70%, 90%, and 100% ethanol, the specimen was placed in acetone for 2 min at room temperature and then air dried. Endogenous peroxidase activity was blocked by dipping the samples in 3% H2O2 in methanol for 5 min. After three washes in 1× PBS for 5 min each, the sections were treated with 100 μl of 1 mg/ml RNase in 2× SSC at 37°C for 60 min. The slides were washed three times in 2× SSC for 3 min each and dehydrated through graded ethanol series.

Hybridization Procedure. Biotinylated α-satellite DNA probes specific for the centromeric regions of chromosomes 7 [p7et 1(D7z1)] and 17 [p17 H8(D17z1)] were obtained from Oncor, Inc. (Gaithersburg, MD). The hybridization solution contained 60% formamide in 2× SSC, 5% dextran sulfate, 1 mg/ml salmon sperm DNA, and 0.8–1.0 ng/ml biotinylated DNA probes. Thirty μl of the hybridization solution were applied to each section and covered with a 22 x 22 mm coverslip, which was then sealed with rubber cement. The probe and target DNA were denatured together at 95°C for 4 min and incubated at 37°C overnight in a sealed wet chamber.

Detection of Hybridized DNA Probes. After overnight incubation, the coverslips were removed and the slides were washed in 50% formamide, 1× SSC (pH 7.0) for 15 min twice at room temperature followed by two 15-min washes in 0.1× SSC at 37°C. After a 10-min preincubation in 3% bovine serum albumin in PBS to block nonspecific binding, 100 μl of 5 μg/μl avidin (Vector Laboratories, Inc., Burlingame, CA) in 3% bovine serum albumin in PBS were added, and the slides were incubated at 37°C for 30 min in a moist chamber. After being washed three times at 37°C in PBS, 100 μl of 5 μg/μl biotinylated anti-avidin D (Vector Laboratories, Inc.) in 3% bovine serum albumin in PBS were applied to the slides and incubated at 37°C for 30 min. Following another series of PBS washes and avidin and anti-avidin incubations, 100 μl of avidin-biotin-peroxidase complex solution (Vectastain ABC kit; Vector Laboratories, Inc.) were added, and the slides were incubated at 37°C for 30 min. After being washed three times with PBS, the hybridized slides were incubated with a 50 ml PBS solution containing 50 mg diamino-benzidine tetrahydrochloride (Sigma), 35 mg of NiCl2, and 10 μl of 30% H2O2 in 50 ml of PBS at room temperature for 2–3 min to allow signal development. The slides were then successively washed in PBS for 5 min, rinsed in deionized running water for 10 min, air dried, and counterstained with Giemsa stain (0.02%) for 10–20 s. The sections were then mounted in Eukitt (Calibrated Instruments, Inc., Hawthorne, NY) and examined under a light microscope. Details of this technique have been described elsewhere (35, 36).

Analysis of Chromosome Copy Number. Areas for analysis were selected by the pathologist by comparing the hybridized slides to a corresponding hematoxylin-eosin-stained adjacent section. Since the centromeric region of a chromosome occupies only a small region of the interphase nucleus, the hybridized signals appear as small dark spots. At least 200 nuclei were scored in each defined histological area. The following scoring criteria (37) were applied for the ISH signals: (a) nuclei should not be covered by cytoplasmic materials; (b) nuclei should not overlap; (c) signal intensity should be more or less of the same homogeneous staining intensity; (d) minor hybridization spots, which can be recognized by a smaller size and lower intensity, should be excluded; (e) signals may only be counted when completely separated from each other; and (f) paired or closely opposed spots should be counted as one signal.

The total number of signal spots was divided by the number of nuclei analyzed to obtain a chromosome index. Because of sectioning artifacts and the fact that hybridization efficiency may vary slightly from one experiment to another, normal diploid lymphocytes present in the same section were used as internal controls. Preliminary experiments had suggested that sectioning artifacts did not significantly favor detection of signals in small cells versus large cells (e.g., lymphocytes versus epithelial cells). Thus it was felt that lymphocytes could also serve as a quantitative internal control. By dividing the chromosome index of the epithelial cells by that of the lymphocytes in the same section, a normalized chromosome index was obtained.

DNA Content Analysis. Adjacent sections were dewaxed and digested with 0.4% pepsin in 0.2 N HCl as described above for 30 min at 37°C. After being washed with deionized water for 5 min three times, the slides were treated for 30 min at 37°C with 2× SSC and washed for 5 min three times with deionized water. Preliminary studies indicated that these treatments lessened the influence of chromatin density differences on DNA quantitation measurements. The samples were then stained with Schiff’s Feulgen stain for 60 min at room temperature, washed with water, and dehydrated through graded alcohols and xylene (38). DNA density measurements were performed using a Joyce Loebl Image Analysis System (Joyce-Loebl, Ltd., Dukesway, England). Lymphocytes in the same sections were used as an internal control for DNA content. Areas for analysis were selected by comparison with a corresponding hematoxylin-eosin-stained adjacent section. Where possible, at least 200 cells in each histological stage were analyzed. The DI of each lesion was calculated by dividing the integrated optical density of epithelial nuclei by that of normal lymphocytes.

Statistical Analyses. Statistical analyses were performed using the Student’s t test and linear and multiple regression analyses.

RESULTS

Patient and Tissue Characteristics. Tumor specimens were obtained from the surgical resections of 25 patients with head and neck squamous cell carcinoma. As shown in Table 1, tumor specimens were obtained from various head and neck sites, including the oral cavity (six patients), oropharynx (six patients), hypopharynx (four patients), and larynx (nine patients). All four tumor stages were included, with a predominance of Stage III and IV tumors. All but one of the patients had a documented smoking history, and all patients were previously untreated.

The 25 tumor specimens were chosen because they demonstrated adjacent normal and/or premalignant lesions in the same tissue section as the tumor (Fig. 1). Of the 25 cases considered, 20 exhibited histologically normal adjacent squamous mucosa, 15 hyperplasia, and 21 dysplasia. All the tumor specimens contained infiltrating small lymphocytes, which could serve as internal controls for hybridization efficiency and sectioning artifacts. For comparison with histologically normal epithelium adjacent to the tumors, five oral mucosa biopsies
were obtained from nonsmoking, cancer-free individuals and served as "normal" controls.

Chromosome in Situ Hybridization: Demonstration of Polysomies in Premalignant Lesions. Positive chromosome signals after nonisotopic in situ hybridization and immunochemical detection appear as dark dots on the interphase nuclei in the tissue section. As illustrated in Figs. 2a and 3a, lymphocytes found within the tumor sections showed 0, 1, or 2 signals/nucleus for chromosomes 7 and 17, respectively. Similarly, normal control epithelium from unaffected individuals showed 0, 1, or 2 signals/nucleus (Figs. 2b and 3b). For whole diploid cells, one would expect to see two signals each for chromosomes 7 and 17. However, 2 or fewer signals/nucleus were observed here because 6-µm-thick sections produced truncated nuclei. Under the conditions used in these experiments, the mean chromosome index for normal diploid lymphocytes was determined to be 1.13 ± 0.02 for chromosome 7 and 1.07 ± 0.08 for chromosome 17 (Table 2). The chromosome number frequencies found in the lymphocytes serve as internal controls for sectioning artifacts as well as variations in hybridization efficiencies from slide to slide.

In contrast to that found in normal control epithelium and in lymphocytes found within the tumor specimens, occasional nuclei within the histologically normal epithelium adjacent to the tumor exhibited 3 copies of chromosome 7 or 17 (Figs. 2c and 3c). Moreover, cells exhibiting 2 or more chromosome copies/cell appeared to increase in frequency as the tissues progressed histologically from normal to dysplasia to dysplasia to carcinoma. A quantitative example of this trend is shown for one patient's tumor in Fig. 4 for both chromosomes 7 and 17.

Evidence for Increased Genetic Alterations during Multistep Carcinogenesis. If head and neck tumorigenesis is a multistep process resulting from an accumulation of genetic changes, one might expect to observe an increased incidence of chromosome polysomy with histological progression from normal to malignant tissue. One parameter that could be used to describe the data is the normalized chromosome index (normalized to that found in lymphocytes within the same tissue section). As shown in Fig. 5 (a and b) for chromosomes 7 and 17, respectively, there was a general trend toward increased chromosome indices as the tissue progressed from normal to hyperplasia to dysplasia to carcinoma. However, there was a considerable range of values within each histological stage such that a significant difference from normal control epithelium was only observed as tissues progressed to the dysplastic stage for chromosome 7 (P = 0.002) and to squamous cell carcinoma for chromosome 17 (P = 0.002) (Table 2). In contrast, when this parameter was used, no significant differences were detected between normal and hyperplastic lesions for chromosome 7 (P = 0.12) and 17 (P = 0.22), respectively.

To determine whether DNA content changes correlated with chromosome changes seen by in situ hybridization, DNA content measurements were made by Feulgen analysis on tissue sections adjacent to those analyzed for chromosome changes. As shown in Table 2, the mean DNA content also increased as tissues progressed from normal to cancerous. While the mean DI did not appear to differ between adjacent normal epithelium (DI, 0.99 ± 0.20) and hyperplasia (DI, 0.99 ± 0.27) (P = 0.396), a significant DNA index increase was observed when tissues progressed from normal to dysplastic (DI, 1.17 ± 0.30) (P = 0.004) and normal to cancerous (DI, 1.35 ± 0.39) (P < 0.005). However, there was no significant difference in DNA content between dysplasia and tumor lesions (P = 0.071). In addition, the coefficient of variation of the tumor cells was higher than that found for internal control lymphocytes, possibly reflecting tumor heterogeneity. However, on a case-by-case basis, the changes in DNA content did not correlate with polysomies of chromosomes 7 and 17.

One problem with expressing the chromosome copy number data as a mean chromosome index is that a significant increase can only occur when there is an outgrowth of a clone carrying multiple copies of a particular chromosome. While the appearance of an outgrowing clone might be an important parameter for assessing risk in a premalignant lesion, the mean chromosome index in the absence of such a clone might give an underestimate of the amount of underlying overall genetic change occurring in a tissue. To better detect more infrequent genetic events in the tumor field, we determined the fraction of cases that exhibited cells with 3 or more copies of a chromosome/cell. In no case did cells from either normal control epithelium or lymphocytes exhibit 3 or more chromosomes 7 or 17 copies. In contrast, in 35% and 40% of 20 cases, histologically normal epithelium adjacent to the tumor showed cells with more than 2 copies of chromosomes 7 and 17, respectively (Fig. 6). These frequencies increased to 66.7% and 46.7%, respectively, for hyperplastic lesions and 95% and 81%, respectively, for dysplastic lesions adjacent to the tumor. Finally, 96% and 96% of the squamous cell carcinoma regions exhibited cells with 3 or more copies of chromosomes 7 and 17, respectively.

The fact that these observations represented somewhat rare events in the premalignant regions is underscored by the finding that the mean fraction of cells exhibiting 3 or more copies of chromosomes 7 and 17/cell was 1% (range, 0.5-2.0%) and 1.5% (range, 1.0-5.0%), respectively, for the histologically normal tissue adjacent to the tumor. Nevertheless, this is different from 0% for normal control epithelium and normal lymphocytes in the tumor region. In hyperplastic regions, the frequencies of cells with 3 or more copies of chromosomes 7 and 17 was 1% (range, 0.5-3%) and 2% (range, 0.5-5.0%), respectively. In dysplastic tissues, these frequencies increased to 3% (range, 0.5-21.0%) and 3.5% (range, 0.5-17.5%), respectively. In contrast, the tumor regions showed means of 8% (range, 2-33.5%) and 5% (range, 0.5-23.0%) cells showing 3 or more copies/cell. While these numbers might seem small even when outgrowth of a polysomie clone took place, it must be remembered that these measurements are being made on formalin-fixed, paraffin-embedded tissue sections in which the nuclei had been truncated. Thus these frequencies were most likely an
underestimation of the occurrence of polysomies in the premalignant field adjacent to the tumor.

Correlation with Clinicopathological Parameters. No correlation was found between the frequencies of cells exhibiting polysomies of both chromosomes 7 and 17, nor was a correlation found between chromosome polysomy and changes in DNA content. The small number of patient samples limits our ability to reach significant conclusions; however, higher copy numbers of chromosome 7

Fig. 1. Light micrographs of histological stages present in head and neck squamous cell carcinoma tissue sections (H&E staining). (a), lymphocytes within the tumor section; (b), normal control mucosal epithelium from cancer-free individual. Histologically normal (c), hyperplastic (d), and dysplastic (e) epithelium is found adjacent to the tumor (f).
were observed in primary tumors with Stage I–III than Stage IV ($P = 0.041$). Patients without nodal metastases had a higher incidence of chromosome 7 polysomy than did patients with nodal metastases ($P = 0.035$); however, this correlation was not observed for chromosome 17 polysomy. No significant correlation was found between chromosome 7 and 17 polysomies, DNA content, and smoking history (pack years) for either premalignant or malignant lesions (multiple regression analysis).
DISCUSSION

The purpose of this study was to gain a better understanding of the genetic basis of head and neck tumorigenesis. While a variety of clinical observations had suggested that head and neck tumorigenesis involves a multistep process occurring in a field exposed to carcinogens, it was of interest to determine whether these processes could...
be correlated with genetic events occurring in the affected tissue regions. To this end, in situ hybridization with chromosome-specific, centromeric DNA probes was used to determine whether chromosome copy number changes could be detected in histologically normal epithelium and premalignant lesions adjacent to carcinomas of the head and neck.

The studies reported here offer two major important findings. First, genetic alterations, visualized as increases in chromosome 7 and 17 copy numbers, were observed in the histologically normal-appearing epithelium adjacent to the tumors in 35% and 40% of the cases, respectively. Such polysomies were not observed in normal “control” epithelium taken from unaffected, nonsmoking individuals or from lymphocytes in the tumor region. This measured incidence of polysomy in the histologically normal-appearing epithelium adjacent to the tumor can really be considered an underestimate of the true polysomy incidence because the data were obtained on tissue sections containing truncated nuclei, and probes for only two chromosomes were utilized in these studies.

The observation of chromosome number abnormalities in the normal and premalignant regions adjacent to the tumor supports the notion of field cancerization. Since this epithelial field harbors genetic alterations, the entire exposed region is at increased risk for developing multiple independent foci of lesions initiated toward malignancy. This would explain the increased risk for the formation of second or multiple primary tumors found in patients with head and neck cancer. Further studies using in situ hybridization will be useful for determining the extent of the genetically altered field in the aerodigestive tract.

The finding of chromosome number changes in the premalignant regions near head and neck tumors is not unexpected. First, most of the agents thought to be carcinogens for the head and neck region are also known to cause chromosome abnormalities (39, 40). In fact, many previous studies have reported increased frequencies of micronuclei at various sites in the aerodigestive tract in both those individuals exposed to various tobacco-related carcinogens and those harboring premalignant lesions (41–44). Second, individuals with head and neck cancer have been shown to demonstrate increased in vitro sensitivity to chromosome-damaging agents, especially those individuals in whom second primaries develop (45, 46). Over the long run, this increased chromosome breakage sensitivity might lead to an accumulation of genetic alterations seen as chromosome polysomies in the heavily exposed epithelial tissue.

A second important finding of this study is that the amount of genetic change in the premalignant tissue adjacent to the tumor (as demonstrated by the increased frequencies of chromosome 7 and 17 polysomies) increased as the tissue progressed from normal to hyperplasia to dysplasia to carcinoma. This finding supports the notion of multistep tumorigenesis in the head and neck region. It should be noted that the present study does not necessarily demonstrate the establishment of clones bearing additional copies of chromosomes 7 or 17 in the premalignant lesions. In fact, no correlation was found between the degree of chromosome polysomy and DNA index, suggesting either that the amount of DNA increase produced by a chromosome addition was not sufficient to detectably change DNA content or that the chromosome changes detected were not yet clonal. Demonstration of clonal development awaits a spatial subset analysis of chromosome copy number within the premalignant epithelium or the use of more specific chromosome or molecular probes. The present studies do suggest, however, that the degree of genetic instability increases with histological stage.

The observation of an accumulation of chromosome anomalies in the premalignant regions surrounding head and neck tumors may help to explain the complex karyotypic patterns found in head and neck tumors as well as other solid tumors. Many cytogenetic studies in solid tumors report highly variable karyotypes. In some cases, it is difficult to identify the common chromosome changes associated with tumor development. These complex karyotypic patterns were previously ascribed to karyotypic instability once the tumor had developed (47). In other cases, the complex karyotypic patterns observed were proposed to be the result of tumor heterogeneity or multiple tumors in the resected tissue (48–50).

The finding reported here that dysplastic tissue harbors nearly the same degree of chromosome polysomy as does the carcinoma region suggests that many of the karyotypic complexities may have occurred prior to carcinoma development. This latter notion is supported by two other observations. First, chromosome changes can be observed in

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Table 2: A comparison of the mean normalized chromosome indices and the DNA indices of epithelium at different stages of tumor development.

<table>
<thead>
<tr>
<th>Histology</th>
<th>NCI 7 ±SD</th>
<th>NCI 17 ±SD</th>
<th>DI ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control epithelium</td>
<td>1.05 ±0.03</td>
<td>1.03 ±0.03</td>
<td>ND*</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjacent normal epithelium</td>
<td>1.03 ±0.09</td>
<td>1.07 ±0.10</td>
<td>0.99 ±0.20</td>
</tr>
<tr>
<td>(n = 20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia (n = 15)</td>
<td>1.09 ±0.08</td>
<td>1.14 ±0.11</td>
<td>0.99 ±0.27</td>
</tr>
<tr>
<td>Dysplasia (n = 21)</td>
<td>1.24 ±0.20</td>
<td>1.19 ±0.12</td>
<td>1.17 ±0.30</td>
</tr>
<tr>
<td>Carcinoma (n = 25)</td>
<td>1.39 ±0.21</td>
<td>1.27 ±0.17</td>
<td>1.35 ±0.39</td>
</tr>
</tbody>
</table>

* NCI, normalized chromosome indices.
* ND, Not done.

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Fig. 4. Chromosome 7 signal copy number distribution for a patient’s tumor sample and its adjacent premalignant lesions. Note the trend for increased chromosome copy numbers as the tissue approaches the squamous cell carcinoma.
might interfere with normal growth regulation and might play a more direct role in tumorigenesis. The in situ hybridization technique on tissue sections will provide a powerful tool for directly exploring correlations between specific genetic events (e.g., polysomy of chromosome 7) and specific phenotypic events (e.g., up-regulation of epidermal growth factor receptor expression) in sections where tissue architecture is retained.

One of the goals of this study was to identify genetic biomarkers that might be useful for assessing risk of tumor development in normal, carcinogen-exposed tissue and in premalignant lesions. The findings of chromosome polysomies in histologically normal epithelium adjacent to head and neck squamous cell carcinomas and an increased degree of chromosome polysomy as the tissue progressed toward malignancy suggest that the measurement of generalized chromosomal polysomy by in situ hybridization might provide such a genetic biomarker. The advantage of such a biomarker is that it permits the sensitive detection of infrequent events (reflecting accumulated genetic damage or genomic instability) that are difficult to detect by bulk analyses (e.g., DNA content analysis). The development of procedures to document clonal outgrowth will further strengthen the value of such a biomarker.

The results reported here were based on studies of premalignant lesions in individuals with 100% risk of developing tumors (i.e., these were premalignant lesions adjacent to the tumor). A working hypothesis for future studies could be that individuals whose normal or premalignant epithelium exhibits the greatest degree of genetic abnormalities might be expected to be at the highest risk for tumor development. Indeed, preliminary retrospective studies on oral premalignant lesions suggest that those individuals exhibiting increased degrees of chromosome abnormalities (detected by in situ hybridization) were at the highest risk for the development of oral cancer (56). The predictive power of this risk assessment approach should be increased as the specific genetic events associated with head and neck short-term cultures of apparently normal epithelial tissue in the region of aerodigestive tumors (20).

The choice of chromosome probes for this study was somewhat arbitrary since the object of the study was to examine generalized genetic changes in the tumor field rather than the development of specific, tumor-associated genetic changes. Nevertheless, the pattern of chromosome changes appeared to be different for chromosomes 7 and 17. For example, chromosome 7 polysomy was significantly higher in dysplastic and malignant lesions compared to normal adjacent and hyperplastic lesions, whereas chromosome 17 polysomies appeared to increase more gradually during the multistage process. This finding might suggest that numerical chromosome 7 changes might beition genetic alteration during the hyperplastic to dysplastic transition of head and neck cancer development. The epidermal growth factor receptor gene is located on chromosome 7 p12–13 (52), and increased epidermal growth factor receptor expression at the RNA and protein level occurs in a majority of head and neck cancers (53–55). Thus, changes in chromosome 7 copy number
tumor development are discovered and probes are developed for use on biopsy material. The development of efficient clinical trials for the prevention of aerodigestive tract cancer is dependent on the identification of individuals at risk who may benefit most by such intervention. At present, subjects for such studies are chosen on the basis of known risk factors such as the presence of premalignant lesions (e.g., oral premalignant lesions, bronchial metaplasia/dysplasia), the occurrence of a primary aerodigestive tract tumor, or a history of significant tobacco/alcohol exposure. However, because these individuals are at only a relatively increased risk for tumor development, these studies require large numbers of subjects in order to define a statistically significant impact of the chemopreventive intervention. Moreover, they require extended exposure. However, because these individuals are at only a relatively increased risk for tumor development, these studies require large numbers of subjects in order to define a statistically significant impact of the chemopreventive intervention. Moreover, they require extended study periods if cancer incidence is the primary end point. The studies under study include biomarkers of dysregulation of proliferation, differentiation, and cell loss in the tissue at risk. The development of such biomarkers would not only be useful for identifying those individuals at highest risk, but since they can be used on small biopsy sections, they can be useful as intermediate markers of response during chemoprevention trials.

ACKNOWLEDGMENTS

We thank Marion Gay, Susan Cwerner, and Shyla Kalapurakal for expert technical assistance; Josephine Niechierl for her help in manuscript preparation; and Dr. Vincent Gregoire for help in the preparation of the figures.

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