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Chromosome Polysomy and Histological Characteristics in Oral Premalignant Lesions[1]

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Abstract

Head and neck tumorigenesis has been postulated to represent a multistep process driven by the accumulation of carcinogen-induced genetic changes throughout the exposed tissue field. To better explore this genetic instability process at the tissue level, 59 regions within 26 biopsy tissue specimens from individuals with oral leukoplakia have been subjected to chromosome 9 *in situ* hybridization analysis, and the degree of chromosome instability was related to known clinical/pathological parameters associated with tumor risk. Whereas chromosome indices were similar between high-risk lesion sites and low-risk lesion sites, high-risk lesions showed higher levels of chromosome polysomy than did low-risk sites [median PIs (polysomy indices), 2.1 *versus* 1.4, respectively]. Similarly, dysplastic regions showed significantly higher chromosome polysomy levels than hyperplastic regions (median PIs, 2.4 *versus* 1.5, respectively). Interestingly, however, hyperplastic regions in the same biopsy as dysplastic regions showed two-times higher polysomy levels than those in biopsies without dysplasia (median PIs, 2.6 *versus* 1.3, respectively), suggesting that chromosome polysomy determinations provide a field measurement for the degree of ongoing genetic insult. Finally, chromosome polysomy tended to persist or increase in the superficial epithelial layers in regions showing koilocytosis, whereas their frequency decreased in nonkoilocytotic regions, suggesting that epigenetic factors may serve to perpetuate the levels of genetically unstable cells in the epithelium. These results provide direct support for the field cancerization process and suggest that measurements of genetic instability might provide additional biological information beyond histology and lesion site characteristics in the assessment of head and neck cancer risk.

Introduction

Despite the introduction of novel therapeutic modalities into the treatment of human head and neck cancer, improvements in long-term survival rates have only been modest (1). As a result, there has been increasing interest in the development of chemopreventive strategies to slow or stop the onset of invasive disease (2, 3). However, such chemopreventive trials have been limited by a number of factors, including the inability to identify high-risk groups and the extended period of risk before cancer onset (4). As a result, chemoprevention trials using cancer incidence as a primary end point require the inclusion of large numbers of subjects and long follow-up times. To overcome these limitations, recent clinical trials have focused on short-term histological and biomarker end points in subjects with modest cancer risk (5, 6). Oral leukoplakia/erythroplakia has been considered a premalignant condition because its presence places the individual at increased cancer risk. However, the overall incidence of cancer development in subjects with oral leukoplakia is in the range of 4.4 to 17.5% in different studies (7, 8). When histological evidence of moderate to severe dysplasia is present, the incidence of cancer development substantially increases to 36% (9). Although histological evidence of epithelial dysplasia is still the most important predicator for cancer risk, epithelial histological appearance is not always predictive of individual patient outcome; *e.g.*, the cancer risk is still significant in subjects with hyperplasia and mild dysplasia, and many individuals with severe dysplasia show persistent stability for many years. Moreover, the site of head and neck cancer frequently occurs away from the oral leukoplakia lesions. Therefore, there is a need for the development of objective biomarkers that can augment histological risk assessment and can provide cancer risk information for the whole tissue field.

Because of its association with tobacco and alcohol exposure, head and neck tumorigenesis has been proposed to represent a multistep process involving the accumulation of genetic alterations in the carcinogen-exposed field (10, 11). This hypothesis is supported by the frequent presence of premalignant lesions in the epithelial field of head and neck cancers and the high frequency of second primary tumors in patients definitively treated for their first head and neck primary tumor (12). To examine the nature of genetic instability in the surrounding tissue fields of head and neck tumors, our group explored previously (13) the use of the technique of CISH[4] to measure the frequency of chromosome polysomy (*i.e.*, the frequency of cells with three or more chromosome copies) in head and neck tumors and their adjacent premalignant lesions. These studies

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[4] The abbreviations used are: CISH, chromosome *in situ* hybridization; P.I., polysomy index; C.I., chromosome index; HPV, human papillomavirus.

demonstrated that chromosome polysomy could be detected in histologically normal epithelium in the field of head and neck tumors and that the degree of genetic instability appeared to increase as the tissue progressed through a histological progression from normal to hyperplasia to dysplasia to tumor. In contrast, chromosome polysomy was rarely observed in normal epithelium of control subjects.

The premalignant lesions adjacent to the cancer described above represented epithelium in a 100% tumor risk field. Therefore, it was of interest to determine whether genetic instability could be detected in oral premalignant lesions in subjects without head and neck cancer. A subsequent limited study of 13 subjects demonstrated that chromosome polysomy could be detected in hyperplastic and dysplastic oral leukoplakia lesions (14). Whereas the degree of evident chromosome polysomy was highly variable between subjects, dysplastic lesions appeared to show higher levels of chromosome instability than hyperplastic lesions. Of interest, three of the four cases exhibiting relatively high levels of chromosome instability in their oral leukoplakia biopsies subsequently developed head and neck cancer. Therefore, these pilot results and a subsequent study (15) suggested that the measurement of generalized chromosome polysomy might provide a genetic marker for assessing the risk of cancer development in subjects with oral leukoplakia.

For a biomarker to be useful for head and neck cancer risk assessment, it should accomplish several goals. First, it should provide information beyond that of histology. Second, it should provide risk information for the whole epithelial tissue field at risk. Third, it should reflect the etiological pathways driving head and neck tumorigenesis such that it could be used as an intermediate marker of chemopreventive effect (16). The goal of the present study was to expand the previous experience of measuring chromosome polysomy in oral leukoplakia lesions using CISH. In particular, we wanted to better determine the relationship between the levels of chromosome polysomy and histological progression, and we wanted to determine whether generalized genomic instability could be detected throughout the biopsy specimen, not only in the most histologically advanced region. The results of this study demonstrate that, whereas dysplastic lesions generally show higher chromosome polysomy levels than hyperplastic lesions, hyperplastic lesions contiguous to dysplastic lesions exhibit similar levels of genetic damage to that of dysplastic lesions. In addition, the presence of koilocytosis was associated with the retention of polysomic cells in the epithelium.

Materials and Methods

Tissue Materials. Oral punch biopsy specimens (4 mm in diameter) of oral leukoplakia or erythroplakia lesions were obtained from 26 subjects participating in an ongoing chemoprevention trial conducted at the M. D. Anderson Cancer Center (17). For entry into the clinical trial, the subjects had to exhibit clinical and histological evidence of oral leukoplakia and/or erythroplakia. Subjects with cancer or prior cancer therapy were excluded. Lesions occurring in the lateral and/or ventral tongue, floor of mouth, or mucosal lip were considered high-risk regions, whereas lesions occurring in the buccal mucosa, gingiva, vestibule, and dorsal tongue were considered low-risk regions. The biopsies were obtained before chemopreventive treatment, fixed in buffered formalin, and embedded in paraffin. Specimens exhibiting tangential cuts or autolysis were excluded from the analysis.

Histological Analysis. H&E-stained slides were reviewed by two pathologists (J. K. and A. E.). To facilitate multiple chromosome instability measurements on each case, two to five epithelial regions were marked on each tissue section according to the size of the tissue and the histological findings. Even sections showing a uniform histology were divided into two or three regions to avoid observer bias during chromosome polysomy analysis. A total of 59 tissue regions were identified from the 26 biopsies. Each region was histologically characterized into one of four categories, *i.e.*, hyperplasia or mild, moderate, or severe dysplasia. Dysplasia was determined according to WHO criteria (18). In addition, the presence or absence of koilocytosis, diagnosed by a distinct perinuclear halo and condensed, pyknotic, or angulated nuclei (19), was noted when present.

CISH. Tissue sections (4 μm) from paraffin blocks of leukoplakia biopsies were placed on silane-coated slides. The slides were placed overnight on a slide warmer at 65°C, then dewaxed in xylene, and cleared in 100% ethanol. The slides were then treated with 1 mg/ml RNase in 2 $\times$ SSC and digested with 0.4% pepsin (Sigma Chemical Co., St. Louis, MO) in 0.2 N HCl as described previously (20).

Biotin-labeled α satellite DNA (0.8 ng/μl), specific for the pericentromeric region of chromosome 9 (Oncor, Gaithersburg, MD), was mixed in a hybridization solution composed of 60% formamide in 2 $\times$ SSC, 5% dextran sulfate, and 1 mg/ml salmon sperm DNA. The hybridization solution was placed on the tissue section, and the two were denatured together at 93°C for 6 min and incubated at 37°C overnight. The next day, the sections were washed in 50% formamide in 1 $\times$ SSC (pH 7.0) at room temperature twice for 15 min each, followed by washing thrice in 0.1 $\times$ SSC at 37°C for 10 min each. The slides were then treated with a 3% BSA-blocking solution for 10 min and then incubated with avidin (Vector, Burlingame, CA) and, subsequently, with antiavidin (Vector). These steps were repeated to amplify the signal, and then an avidin-biotin-peroxidase complex solution was applied. Each incubation step was carried out at 37°C for 30 min, followed by a wash with PBS. The hybridization signals were developed with 50 mg of diaminobenzidine tetrahydrochloride (Sigma) and 35 mg of $NiCl_2$ in 100 ml of PBS and counterstained with Giemsa.

Analysis of Chromosome Signals. The criteria for the scoring of chromosome hybridization signals were as described previously (20). The levels of chromosome hybridization signals in the lymphocytes infiltrating the submucosa served as controls for determining the hybridization efficiencies in the epithelial layers. Chromosome copy numbers were assessed separately in the basal, parabasal, and superficial cell layers to determine whether the detected degree of genetic instability was influenced by cell maturation or location in the epithelium. From 100 to 900 cells were counted in each predetermined region at $\times$1000 magnification. The CI^4 for each region was calculated by dividing the total number of signals detected by the total number of nuclei scored. The chromosome P.I. was defined as the percentage of scored nuclei exhibiting three or more chromosome copies. Tissue sectioning results in the truncation of nuclear material and leads to an under-representation of chromosome copy number. To avoid bias, nuclei exhibiting no signals were included in all of the calculations.

The Magiscan Image Analysis system (Joyce Loebl, Ltd., Dukesway, England) was used to record the spatial distribution of chromosome counts in the epithelium (21). The relative x- and y-coordinates and detected chromosome copy number of each scored nucleus was recorded in list mode. This permitted the preparation of a genetic map of the tissue section where each nucleus is represented by a dot, the color of which represents the chromosome copy number detected for that cell.

Table 1 Patient characteristics ($n = 26$)

Age (range)	50 (27–78)
Gender	
Male	18
Female	8
Lesion site	
Tongue	10
Floor of mouth	3
Buccal mucosa	5
Gingiva	4
Vestibule	2
Mucosal lip	2
Smoking history	
Present	20
Absent	6

Statistical Analysis. The data collected did not show a normal distribution; therefore, statistical analysis of the data was carried out using nonparametric methods. The levels of genetic instability between histological and high-risk *versus* low-risk groups were compared using the Mann-Whitney test. The Wilcoxon signed rank test was used to compare the differences in P.I. between basal/parabasal cell layers and the superficial epithelial layers in each region.

Results

Clinical and Histological Characteristics. Twenty-six biopsy specimens from 26 cases were available for both clinical review and analysis of genetic instability by CISH. As shown in Table 1, the median age of the subjects was 50 years of age, ranging from 27 to 78 years. Eighteen of the cases were male, with a median age of 57, whereas 8 cases were female, with a median age of 45.5. Twenty of the 26 cases had a documented significant smoking and drinking history. The remaining cases reported a negative smoking history; however, four of these six cases did report a drinking history. The oral premalignant lesions were located in various sites, including the lateral and/or ventral tongue (nine cases), gingiva (four cases), buccal mucosa (five cases), floor of mouth (three cases), vestibule (two cases), mucosal lip (two cases), and dorsal tongue (one case). Of the 59 regions identified on the tissue sections that had adequate *in situ* hybridization signals, 47 regions showed hyperplasia whereas 12 regions showed dysplasia (7 mild, 1 moderate, and 4 severe). Fifteen of the hyperplastic regions examined occurred in the same biopsy as documented dysplasia, *i.e.*, either in the same tissue section or on another tissue section deeper into the biopsy specimen. In 14 biopsies taken from individuals with leukoplakia in high-risk sites (*i.e.*, lateral and/or ventral tongue, floor of mouth, or mucosal lip), 7 (50%) showed dysplasia somewhere in the biopsy. In contrast, in 12 biopsies taken from individuals with leukoplakia in low-risk sites (*i.e.*, the buccal mucosa, gingiva, vestibule, or dorsal tongue), only 3 biopsies (25%) showed evidence of dysplasia (Table 2).

Chromosome Polysomy and Lesion Site. As shown in Fig. 1, the chromosome hybridization signals appear as dark spots associated with the nuclei. Whereas most of the nuclei showed zero, one, or two signals/cell, in some cases, cells exhibiting three or more signals were apparent in the epithelial cell layers. Such cells were rarely observed in the stromal fibroblasts or infiltrating lymphocytes. The CIs of the lymphocyte controls ranged from 1.19 to 1.52 (median, 1.38), suggesting that the efficiency of hybridization can vary somewhat from one reaction to another. As shown in Table 2, the CIs of the basal/parabasal epithelial layers were similar between the high-risk and low-risk sites. This suggests that the overall C.I. of a region might not be a sensitive biological end point for describing genetic instability in the tissue. This observation is in line with previous observations (22) that a significant change in the C.I. can only be detected when more that 25% of the population is composed of monosomic or trisomic cell populations. On the other hand, high-risk sites showed significantly higher frequencies of chromosome polysomy than that of low risk sites (Table 2). In particular, the median (range) P.I. of high-risk sites was

Table 2 Relationship between histology and genetic instability in high- and low-risk epithelial sites

	High-risk sites ($n = 34$)	Low-risk sites ($n = 25$)
Histology		
Hyperplasia	27	20
Mild dysplasia	7	0
Moderate dysplasia	0	1
Severe dysplasia	0	4
C.I.		
Median (range)	1.4 (1.0–1.6)	1.4 (1.1–1.5)
P.I.		
Median (range)	2.1 (0–9.3)	1.4 (0–4.2)

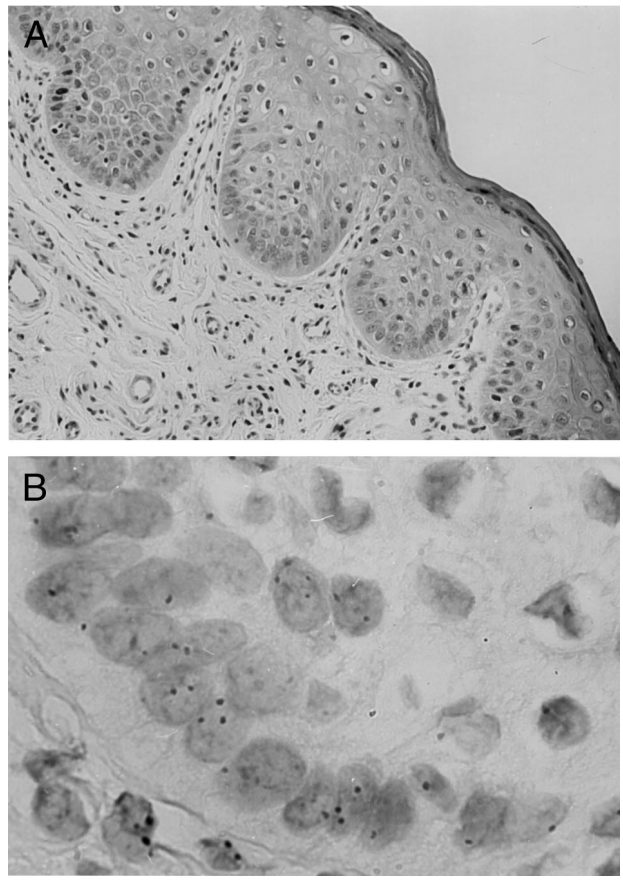

Fig. 1. Detection of chromosome polysomy in leukoplakia lesion showing mild epithelial dysplasia by CISH. *A*, H&E of epithelium showing mild dysplasia (×200); *B*, CISH of the same area of Fig. 1*A* illustrating cells with three signals/cell scattered in the basal and parabasal cell layers (×1000).

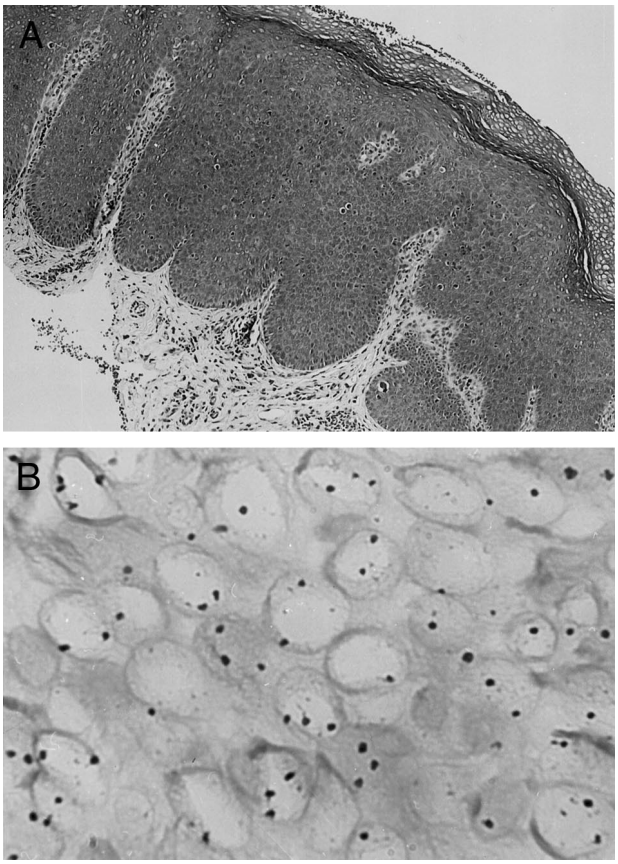

Fig. 2. Detection of clusters of cells within the leukoplakia epithelium showing chromosome polysomy. *A*, H&E of epithelial lesion showing severe dysplasia (×100); *B*, CISH analysis of the same area shown in Fig. 2*A* showing a cluster of cells with three chromosome 9 copies/cell (×400).

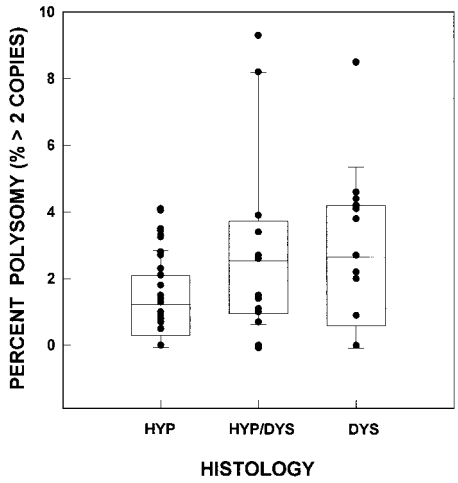

Fig. 3. Comparison of chromosome polysomy levels in epithelial lesions as a function of histology. Note that hyperplastic lesions in the same tissue field as dysplasia show elevated chromosome polysomy levels closer to that of dysplastic lesions than hyperplasia lesions alone.

2.1 (0–9.3) compared with values of 1.4 (0–4.2) in the low-risk sites ($P = 0.147$ by the Mann-Whitney test). Thus, whereas high-risk sites tended to exhibit higher degrees of genetic instability than did low-risk sites, considerable variability in chromosome polysomy was observed within groups with different risks based on the site of disease.

Chromosome Polysomy and Histology. In general, dysplastic regions showed a tendency for higher degrees of polysomy than did regions exhibiting hyperplasia [median (range) PIs of 2.4 (0–8.5) *versus* 1.5 (0–9.3), respectively; $P = 0.135$]. Moreover, the median P.I. of four regions showing severe dysplasia was 4.0 (range, 2.9–4.2), whereas the median P.I. for the seven lesions with mild dysplasia was 1.6 (range, 0–8.5). One lesion with moderate dysplasia associated with lichenoid inflammation had a P.I. of 1.9. In most cases, the cells showing three chromosome copies were randomly scattered throughout the epithelial layers. However, in some cases with higher polysomy levels, there appeared to be a clustering of cells with three or more chromosome copies, perhaps suggesting the presence of a localized clonal outgrowth (Fig. 2).

A considerable overlap of polysomy values was observed between the various histological groupings. To better understand the biological basis for this variability, the hyperplastic lesions were divided into those that existed in the presence of dysplasia elsewhere in the biopsy specimen (*e.g.*, adjacent or deeper in the biopsy) and those where hyperplasia represented the highest degree of histological progression in the biopsy specimen (Fig. 3). The median P.I. of hyperplastic lesions in the same biopsies as dysplastic lesions was 2.6 (range, 0–9.3) compared with a median P.I. of 1.3 (range, 0–4.1) in lesions where hyperplasia represented the most advanced stage. This difference was statistically significant by the Mann-Whitney test ($P = 0.0465$). Thus, hyperplastic lesions in the field of dysplastic lesions show chromosome instability characteristics closer to those of lesions showing dysplasia. Such an example is shown in Fig. 4 where both the hyperplastic region and the focally dysplastic region showed similar levels of chromosome polysomy.

Chromosome Polysomy and Koilocytosis. During the course of this study, a number of regions were noted to exhibit koilocytosis. To determine whether koilocytosis was associated with genetic instability, the levels of chromosome polysomy were compared in lesions showing koilocytosis with those not showing koilocytosis. Because koilocytosis is usually found in the more superficial epithelial layers, the levels of chromosome polysomy were also compared between the basal/parabasal layers and the superficial layers in each region. A total of 59 epithelial regions from 26 biopsies were evaluable for polysomy indices in both basal/parabasal and superficial layers. Of these 59 regions, 23 showed signs of koilocytosis and 36 did not. The mean polysomy levels in the basal/parabasal and the superficial layers of regions showing koilocytosis were 1.6 and 1.6, respectively, as compared with 2.5 and 1.5, respectively, for the regions not showing koilocytosis. The higher levels of chromosome polysomy in the non-koilocytotic regions were associated with an increased frequency of dysplastic lesions in this group compared with the koilocytotic lesions. On a region-by-region basis (Fig. 5), the mean difference in P.I.s between the superficial and basal/parabasal layers in regions with koilocytosis was −0.017, whereas that in regions without evident koilocytosis was −0.94%. Moreover, 9 of the 23 (39.1%) regions with koilocytosis showed an increase of chromosome polysomy of greater than 0.5% in cells moving into the superficial layers, compared with only 5 of 36 (13.8%) of the regions without koilocytosis. Thus, whereas the regions showing koilocytosis tended to exhibit less overall chromosome polysomy than regions not showing koilocytosis, they tended to exhibit the same or even higher frequencies of chromosome polysomy in the

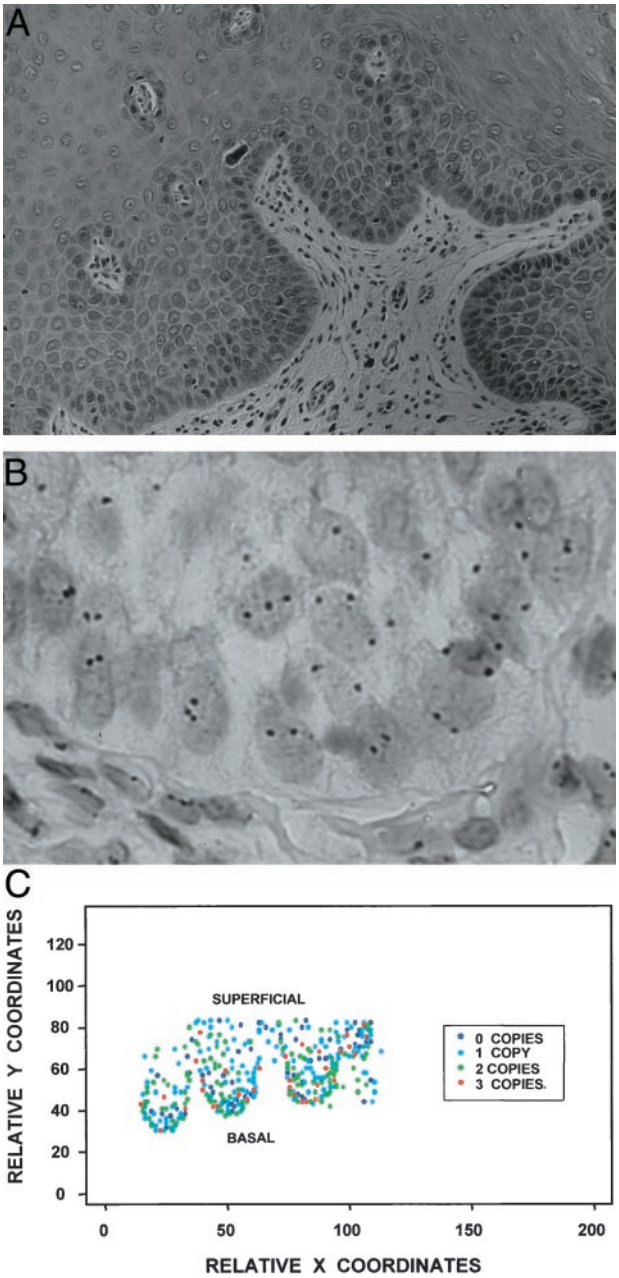

Fig. 4. Detection of high chromosome polysomy in hyperplastic regions of biopsies containing dysplasia. *A*, H&E showing an epithelial region of hyperplasia adjacent to dysplasia; *B*, CISH analysis of the marked hyperplastic region of Fig. 3*A* showing frequent cells with three chromosome 9 signals in the basal and parabasal layers (×1000); *C*, chromosome copy map representation of the same area shown in Fig. 4*B* demonstrating the epithelial location of cells with three chromosome 9 signals (*red-colored dots*). Note the preferential localization of polysomic cells in the basal and parabasal epithelial layers.

more superficial layers as compared with the basal/parabasal layers. This phenomenon was documented using the Magiscan Image Analysis system to generate chromosome copy spatial distribution maps. As shown in Fig. 6*A*, cells exhibiting three or more chromosome signals were dispersed throughout the epithelial thickness with koilocytosis. In contrast, the cells showing polysomy were preferentially distributed in the basal and parabasal layers in the regions not showing koilocytosis (Fig. 6*B*).

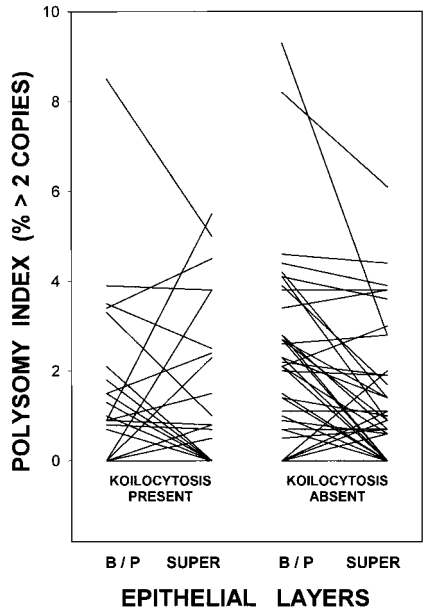

Fig. 5. Comparison of chromosome polysomy levels between basal/parabasal and superficial epithelial layers in lesions exhibiting (*left*) or not exhibiting (*right*) koilocytosis. Note the more frequent retention of cells exhibiting chromosome polysomy into the superficial epithelial layers in lesions with koilocytosis.

Discussion

The risk of cancer development in subjects with oral premalignant lesions is traditionally estimated by several factors including the presence and severity of epithelial dysplasia, smoking or alcohol exposure history, evidence for viral infection, and sites and patterns of the lesions. In particular, lesions of the floor of mouth, lip, and tongue are considered relatively high risk because epithelial dysplasia and subsequent cancer development are more frequent than in other sites (23–26). Moreover, it has been postulated that these sites are at higher risk because of either the pooling of soluble carcinogens in the "sump" of the floor of the mouth or the association with actinic damage to the lip (25, 26). Because cancer development is postulated to be driven by the accumulation of genetic errors in the target tissue (27–29), it was of interest to determine whether hallmarks of genetic instability such as chromosome polysomy could be detected at higher frequencies in tissues thought to be associated with higher risk for cancer development.

The results reported in this study (Table 2) demonstrated that the levels of chromosome polysomy were higher in biopsies from "high-risk" sites compared with those taken from "low-risk" sites. Whereas this might have been expected on the presence of a higher frequency of dysplastic lesions in the high-risk sites, this difference in chromosome polysomy levels between high- and low-risk sites remained when only hyperplastic lesions were considered. Nevertheless, a considerable degree of overlap of chromosome polysomy levels existed between high- and low-risk lesions, suggesting that measures of genetic instability might provide additional risk information to that of site location.

The results reported in this study also demonstrated that, whereas the levels of chromosome polysomy were generally higher in dysplastic epithelium than in hyperplastic epithelium, a high degree of variation of chromosome polysomy was detectable within each histological class. However, hyperplastic

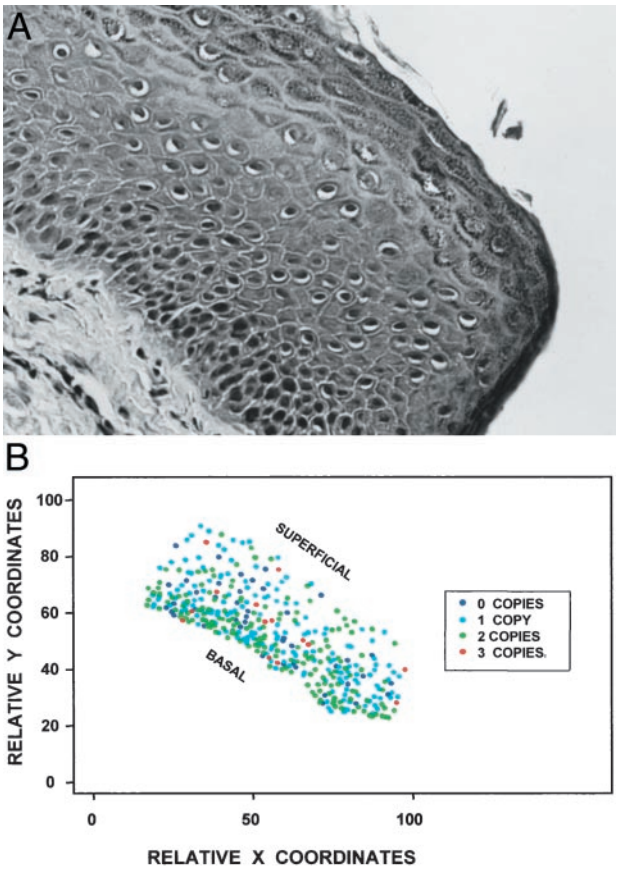

Fig. 6. Example of retention of cells with chromosome polysomy into the superficial epithelial layers of a koilocytotic lesion. *A*, H&E of a koilocytotic area showing pyknotic nuclei and perinuclear halos (×400); *B*, chromosome copy map of the same area shown in Fig. 6A demonstrating the presence of polysomic cells (*red dots*) dispersed throughout the epithelial thickness.

regions in biopsies that also showed dysplasia in another location exhibited chromosome polysomy levels more comparable with those found in dysplasia. These levels were considerably higher than those found in hyperplastic regions in biopsies without evident dysplasia. These results suggest that measurements of chromosome polysomy might reflect the ongoing process of generalized genetic instability in the carcinogen-exposed tissue and, therefore, might provide risk assessments that are somewhat independent of the histological appearance of that particular lesion. Indeed, head and neck tumorigenesis has been proposed to represent a field cancerization process whereby a whole tissue field is exposed to carcinogen insult and is at increased cancer risk throughout the field.

One of the issues facing the use of biomarkers for head and neck cancer risk assessment is knowing the most informative site for biopsy. Whereas individuals with oral premalignant lesions in high-risk sites are known to be at increased risk for cancer development and whereas the finding of epithelial dysplasia is associated with increased cancer risk, the cancers that do subsequently develop are frequently located away from the site of the premalignant lesion. Thus, biomarkers are needed that can provide risk information from assessments of random biopsies from the tissue at risk. Our previous studies (13) demonstrated that normal and premalignant lesions adjacent to head and neck cancers exhibited significant degrees of genetic instability when assayed by CISH. The present study reinforces our prior observations that significant degrees of chromosome polysomy can also be found in premalignant lesions in subjects at increased cancer risk (14, 15). Moreover, the finding of increased chromosome polysomy levels in hyperplastic lesions in the field of dysplastic lesions further supports the possibility that chromosome polysomy measurements can provide a more global assessment of the forces driving the tumorigenesis process in the tissue field and thus provide additional risk information to lesion site and histology.

A number of driving forces are likely to be involved in head and neck tumorigenesis, including the degree of tobacco and alcohol exposure. Unfortunately, the number of cases examined in this study was not enough to explore the relationship between carcinogen exposure and the degree of ongoing chromosome polysomy. However, oral leukoplakia is associated with a number of identifiable or unidentifiable factors in addition to tobacco or alcohol exposure, including tissue irritants, nutritional deficiencies, and viral infections. Whereas these associated factors may or may not induce genetic instability on their own, they may play a synergistic role in the accumulation of genetic damage by promoting cell proliferation or preventing the loss of genetically altered cells. During the course of this study, a number of koilocytotic regions were detected in the biopsied lesions. Koilocytosis is normally found in association with HPV infection. However, the role of HPV infection in the etiology of head and neck cancer is still uncertain (19, 30–32). Unfortunately, insufficient biopsy material was available from these biopsies to assess HPV status or HPV-associated gene expression.

Nevertheless, the results reported in this study suggest that although koilocytotic lesions did not necessarily exhibit increased overall levels of chromosome polysomy, they were associated with a different spatial pattern of chromosome polysomy within the epithelium. In particular, the levels of chromosome polysomy in koilocytotic lesions frequently remained the same or increased in the superficial layers when compared with the basal/parabasal layers. In nonkoilocytotic lesions, the levels of chromosome polysomy appeared to decrease as cells moved into the superficial layers. The reason for these different patterns of chromosomal polysomy in koilocytotic lesions compared with nonkoilocytotic lesions is not well understood. However, a number of possible explanations exist. Most carcinogens are known to induce chromosome damage in exposed cells (33), leading to the up-regulation of cell cycle checkpoints that would either slow subsequent cell growth (34) or induce cell loss of the damaged cells (35). However, the HPV E6 gene product is known to target p53 for proteosome-mediated degradation (36–38), and the HPV E7 gene product is known to inactivate retinoblastoma protein function (39–41). Thus, inactivation of these checkpoint controls in HPV-associated koilocytotic lesions might result in the retention of genetically damaged cells into the superficial layers of the epithelium (42, 43). Indeed, our group has also found that altered p53 expression in oral premalignant lesions is associated with increased chromosome polysomy (44). Alternatively, HPV-associated E6 and E7 expression might cause genetic instability through dysregulation of centrosomal duplication, leading to mitotic errors (45). Thus, HPV infection or other factors that mimic HPV-induced dysfunction (*e.g.*, disruption of p53 function) might interact synergistically with chronic carcinogen exposure to promote chromosome instability in the epithelium.

In summary, the results of this study suggest that measures of ongoing genetic instability such as chromosome polysomy might provide additional insight into head and neck cancer risk assessment beyond that of the location and histology of the

premalignant lesion. The finding that hyperplastic lesions in the field of dysplastic lesions show relatively high levels of chromosome polysomy suggests that this assay measures the level of ongoing genetic instability in the tissue field at risk and thus is an indication of the forces that drive head and neck tumorigenesis. Furthermore, the finding that chromosome polysomy is propagated in koilocytotic epithelium suggests that epigenetic factors might synergistically interact with extrinsic carcinogenic exposure in the accumulation of genetic hits important for tumor development. Additional long-term follow-up studies are required to determine whether the levels of chromosome polysomy in oral premalignant lesions add significantly to the determination of head and neck cancer risk.

Acknowledgments

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