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Chromosome Instability in Lymphocytes: A Potential Indicator of Predisposition to Oral Premalignant Lesions[1]

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ABSTRACT

Oral premalignant lesions (OPLs) are related to tobacco use and mark individuals at high risk for oral cancer development. Increased mutagen sensitivity as measured by an *in vitro* mutagen challenge assay has been shown to be a risk factor for upper aerodigestive tract cancers. In this case control study, we used two assays with mutagens relevant to tobacco exposure (benzo[*a*]pyrene diol epoxide (BPDE) and bleomycin) to see whether sensitivity to these mutagens could be used as biomarkers for assessing risk of premalignant lesions. Furthermore, we evaluated whether 3p21.3 is a molecular target of BPDE damage in lymphocytes of patients with OPLs. There were 82 patients with OPLs and 89 healthy controls frequency matched to the cases on age, sex, ethnicity, and smoking status. These subjects' lymphocytes were treated in two separate experiments with either 2 μM BPDE for 24 h or 0.03 units/ml bleomycin for 5 h, and the frequency of induced chromatid breakage in Giemsa-stained preparations was determined. BPDE-induced 3p21.3 aberrations were scored by fluorescent *in situ* hybridization technique in 1000 interphases/sample. We found that the mean BPDE-induced chromatid breaks per cell were higher in cases than controls (1.05 ± 0.40 and 0.55 ± 0.27, respectively; $P < 0.01$). Similar results were evident with bleomycin-induced chromatid breaks per cell (0.78 ± 0.37 and 0.57 ± 0.31, respectively; $P < 0.01$). After adjusting for age, sex, ethnicity, and smoking status, significantly elevated odds ratios (95% confidence interval) for OPL risk were noted for BPDE sensitivity [12.96 (5.51, 30.46)] and bleomycin sensitivity [3.33 (1.64, 6.77)]. When subjects were categorized into quartiles of the number of breaks per cell, a dose response was observed for both assays. The adjusted odds ratios for subjects with increasing numbers of breaks per cell in quartiles were 2.34, 9.14, and 54.04 for BPDE sensitivity and 1.92, 3.33, and 7.15 for bleomycin sensitivity, respectively. Subjects sensitive to both mutagens had a 50-fold increased risk for OPLs. In addition, there were significantly more BPDE-induced chromosome aberrations at the 3p21.3 locus in cases (51.13/1000) than in controls (40.93/1000; $P < 0.0001$). However, no such difference was observed for 3q13, a control locus. BPDE-induced 3p21.3 aberrations were associated with an elevated risk for OPLs of 6.08 (2.57, 14.4). The degree of BPDE sensitivity at 3p21.3 and risk for OPLs increased in a dose-dependent manner. In summary, BDPE sensitivity and bleomycin sensitivity appear to be individually and jointly associated with elevated risk of OPLs. Furthermore, 3p21.3 may be a molecular target of BPDE in OPLs. This is the first study to examine mutagen sensitivity in a premalignant condition. The next step is to correlate these findings in surrogate (lymphocyte) tissue with molecular events in the target tissue.

INTRODUCTION

OPLs[3] are white (leukoplakia) or red (erythroplasia) mucosal patches in the oral cavity or oropharynx (1). OPLs are associated epidemiologically, geographically, and clinically with oral cancer (1–3). Individuals with these lesions are at high risk for developing oral cancer. Tobacco smoking and alcohol drinking are established risk factors for OPLs (4–6). However, it is unclear why only a fraction of individuals who use tobacco or alcohol develop these lesions or subsequently develop oral cancer. Substantial evidence suggests that the carcinogenic process is driven by the interaction between exposure to exogenous carcinogens and inherent genetic susceptibility. However, little is known about the genetic basis for susceptibility to premalignancy.

Hsu (7) hypothesized that genetic instability modifies an individual's susceptibility to cancer. This notion was supported initially by the rare autosomal recessive disorders AT and XP, which are associated with *in vivo* and *in vitro* chromosomal instability, defective DNA repair, and increased cancer risk. In response to environmental exposures, genetic damage accumulates more quickly in individuals with genetic susceptibility to DNA damage than in those without such instability but with a similar exposure. Consequently, individuals with genetic instability might be at a greater risk for developing cancer. For this reason, the mutagen sensitivity assay was developed as an indirect measure of an individual's DNA repair capacity and, thus, cancer risk (8). Many studies have indicated that mutagen sensitivity is a promising predictor of environment-related cancer risk (8–21). Different carcinogens or mutagens may act on cells through different molecular mechanisms and accordingly may activate different repair pathways. Persons with one DNA repair defect may be sensitive to one mutagen but not to another (22). This assay has been adopted to test a range of different mutagens. In this study, we used the initial test mutagen, bleomycin, and BPDE (a metabolic product of BPDE, which is a component of tobacco smoke) as a more relevant tobacco-specific risk measure (15, 19, 22). BPDE forms covalent DNA adducts requiring nucleotide excision repair (23, 24). Bleomycin is also relevant to tobacco use because it is similar to numerous compounds in tobacco smoke known to cause oxidative damage and is repaired by the base excision and recombination repair systems (25, 26). Persons who are sensitive to both mutagens may have defects in multiple DNA repair pathways.

Recent evidence also suggests that the mutagen-induced chromosome damage is not distributed randomly but rather reflects the susceptibility of specific loci to damage caused by carcinogens (27). The short arm of chromosome 3 may be a prime spot for such damage. Deletion of 3p is the most frequently identified aberration in oral cancer tissue (28, 29), and 3p21.3 has been shown to have tumor suppressor activity (30, 31). Furthermore, 3p deletion occurs more frequently in tumor tissues of patients who smoke than in those who do not (32).

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[3] The abbreviations used are: OPL, oral premalignant lesion; AT, ataxia telangiectasia; XP, xeroderma pigmentosum; BPDE, benzo[*a*]pyrene diol epoxide; PBL, peripheral blood lymphocyte; OR, odds ratio; CI, confidence interval.

This study had several objectives. The first goal was to ascertain whether the sensitivity of cultured PBLs to BPDE or bleomycin can be used as a biomarker to assess risk of OPL. The second objective was to determine whether BPDE-induced chromosome aberrations at 3p21.3 in PBLs were more common in OPL patients than in healthy controls. We hypothesized that the OPL patients would be more likely than controls to exhibit bleomycin and BPDE sensitivity, that using the two tobacco mutagen sensitivity assays in parallel might improve our ability to identify individuals at high risk for OPLs, and that 3p21.3 is a molecular target of carcinogens in tobacco smoke.

PATIENTS AND METHODS

Study Subjects. Participants for this study were identified at The University of Texas M. D. Anderson Cancer Center during 1997–2000 before they were randomized into a chemoprevention trial, and our analyses were conducted in baseline samples taken before the intervention. The inclusion criteria for the 82 cases were the presence of histologically confirmed OPL and age ≥18 years. Patients with acute intercurrent illnesses or infections were excluded, as were those who had had retinoid or carotenoid therapy within 3 months before study entry. A previous study did not show a relationship between ascorbic acid intake and mutagen sensitivity (33). A self-administered questionnaire was used to gather epidemiological data, including recent and prior tobacco for the cases. Before the subjects were randomized into the chemoprevention trial, blood was obtained in heparinized tubes for cytogenetic and molecular genetic analyses. The 89 controls with no history of cancer were identified from the rosters of the largest multispecialty managed maintenance organization in Houston, Texas from an ongoing case control study (19). The controls were matched to the cases by age (±5 years), sex, ethnicity, and smoking status. Questionnaire data were obtained through personal interview for the controls. Laboratory personnel were blinded to each participant's case control status or study status, because the blood samples were coded with Lab ID. Informed consent was obtained from each study subject before enrollment.

Mutagen Sensitivity Assays. One ml of each fresh whole blood sample was added to 9 ml of RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 20% fetal bovine serum, 2 mM L-glutamine, 50 units/ml penicillin, 100 μg/ml streptomycin (Sigma Chemical Co., St. Louis, MO), and 1.25% (volume for volume) phytohemagglutinin (Wellcome Research Laboratories, Research Triangle, NC). After incubation at 37°C with 5% CO_2 for 72 h, the cultures were assessed by the BPDE and bleomycin sensitivity assays. To test for BPDE sensitivity, 40 μl of 0.5 mM BPDE (Midwest Research Institute, Kansas City, MO) were added to the blood cultures to a final concentration of 2 μM. The cultures were then incubated for 24 h. BPDE stock solution was made by dissolving BPDE in tetrahydrofuran (Sigma Chemical Co.) to a concentration of 12 mM. Before adding to the culture, BPDE stock solution was diluted with DMSO to 0.5 mM. For the bleomycin sensitivity assay, bleomycin (Nippon Kayaku Co., Ltd., Tokyo, Japan) was added to the blood cultures to a final concentration of 0.03 units/ml, and the cultures were incubated for 5 h. During the last hour of bleomycin and BPDE treatment, 0.04 μg/ml colcemid was added to the cells to arrest them in mitosis. Then the cells were harvested and stained with Giesma. For each sample, the chromosome breaks in 50 metaphases were counted since a previous study showed that the conventional method of scoring 50 metaphases was adequate (34). The mean number of breaks per cell was used to represent the chromosome breaks of each sample. Chromatid gaps or attenuated regions were disregarded; only frank chromatid breaks or exchanges were recorded. The slides were coded to ensure that laboratory personnel could evaluate them without knowledge of the subjects' case or control status.

Fluorescence *in Situ* Hybridization Analysis of Chromosomal Aberrations. Aberrations were detected by fluorescent *in situ* hybridization with a 3p21.3-specific DNA probe kindly provided by Dr. D. Randle (The University of Texas Health Science Center, Dallas, TX). The 3p21.3 probe is ~145 kb and harbors the *LUCA12*, *LUCA14*, and *LUCA17* genes. Another probe of ~100 kb for chromosome 3q13 was obtained from the Cedars-Sinai Medical Center Collection as an internal control. The 3p21.3 and 3q13 probes were labeled with spectrum green and spectrum orange dUTP (Vysis) by nick translation, respectively, following the manufacturer's instructions.

Dual color fluorescent *in situ* hybridization was performed with LSI hybridization buffer (Vysis) according to the manufacturer's instructions. Briefly, 10 μl of the labeled probe were added to each interphase smear. HYBrite was used to denature the cells at 74°C for 4.5 min and hybridize them at 37°C overnight. The posthybridization washes were in 50% formamide plus 2 × SSC for 10 min at 43°C three times followed by 2 × SSC for 10 min at 43°C and 2 × SSC plus 0.1% NP40 for 5 min at 43°C. Finally, the cells were counterstained with 4′,6-diamidino-2-phenylindole II and viewed through a fluorescent microscope.

3p21.3 aberrations were scored in 1000 interphases/sample. The criteria for scoring fluorescent *in situ* hybridization signals were as follows: (*a*) the nuclei did not overlap; (*b*) signal intensity was about uniform; (*c*) minor hybridization spots smaller and less intense than real signals were excluded; (*d*) signals that were completely separated from each other were counted; and (*e*) paired or close signals were counted as one signal. The most common abnormal event was one signal reflecting a deletion of a specific chromosomal region (Fig. 1).

Statistical Analyses. Pearson's χ^2 test was used to examine the distribution of sex and smoking status between cases and controls. Student's *t* test was used to analyze continuous variables with normal distribution, such as age and cigarette pack-years smoked. Wilcoxon's rank-sum test was used to analyze continuous variables with non-normal distribution, such as number of chromatid breaks and number of 3p21.3 and 3q13 aberrations without data transformation. BPDE sensitivity, bleomycin sensitivity, and BPDE-induced 3p21.3 and 3q13 aberrations were analyzed as continuous variables. They were also dichotomized based on the value of the 75th percentile in the controls as reported previously (27) and also stratified into quartiles based on the number of breaks per cell and aberrations per 1000 interphases in the controls. An individual was considered sensitive to BPDE or bleomycin if the number of breaks per cell was ≥75th percentile of breaks per cell in the controls. Age was dichotomized based on the median age of the controls. Smoking status was stratified into three categories: (*a*) never smoker; (*b*) former smoker; and (*c*) current smoker. Individuals who had smoked <100 cigarettes in their lifetimes were defined as never smokers; those who had stopped smoking 1 year before being diagnosed with OPLs (for cases) or being recruited into the study (for controls) were defined as former smokers. Crude and adjusted ORs and 95% CIs were calculated by univariate and multiple covariate analyses, respectively. All statistical tests were two sided. Age, sex, ethnicity, and smoking status were possible confounders that were adjusted for in the multiple covariate logistic regression.

RESULTS

Table 1 summarizes select characteristics of the cases and controls. The study population included 171 participants (82 cases and 89 controls). The mean age was 57.3 years for the cases and 57.5 years for the controls. The controls were also well matched to the cases in terms of age, sex, ethnicity, and smoking status.

BPDE sensitivity data were available for 71 cases and 71 controls. When the data were dichotomized at 0.74 breaks/cell (the 75th percentile in the control population), 77.5% of the cases exhibited BPDE sensitivity (Table 2). The mean BPDE-induced chromatid breaks per cell were 1.05 ± 0.4 for the cases and 0.55 ± 0.27 for the controls

Table 1 *Distribution of select characteristics*

	Cases		Controls		*P*
	n	(%)	*n*	(%)	
Overall	82	(47.95)	89	(52.05)	
Sex					
Male	45	(54.88)	48	(53.93)	
Female	37	(45.12)	41	(46.07)	0.90
Ethnicity					
Caucasian	72	(87.80)	79	(87.76)	
Mexican-American	6	(7.32)	7	(7.87)	
African-American	4	(4.88)	3	(3.37)	0.88
Smoking status					
Never	16	(20.00)	10	(11.23)	
Former	38	(47.50)	52	(58.43)	
Current	26	(32.50)	27	(30.34)	0.21
Mean age in years (±SD)	57.3	(±12.8)	57.5	(±10.0)	0.91

Table 2 *Risk estimates for BPDE and bleomycin sensitivity*

Mutagen	Cases	Controls	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
	n (%)	*n* (%)	(95%) CI	(95% CI)
BPDE				
Dichotomized (breaks/cell)[b]				
<0.74	16 (22.54)	53 (74.65)	1.00	1.00
≥0.74	55 (77.46)	18 (25.35)	10.12 (4.68–21.90)	12.96 (5.51–30.46)
By quartiles (breaks/cell)[c]				
<0.34	1 (1.41)	15 (21.13)	1.00	1.00
0.34–0.49	4 (5.63)	20 (28.17)	3.00 (0.30–29.66)	2.34 (0.21–26.20)
0.49–0.73	11 (15.49)	18 (25.35)	9.17 (1.06–79.39)	9.14 (1.01–82.58)
≥0.74	55 (77.47)	18 (25.35)	45.83 (5.65–371.69)	54.04 (6.43–454.23)
Trend test			$p < 0.01$	$p < 0.01$
Bleomycin				
Dichotomized (breaks/cell)[b]				
<0.76	40 (49.38)	62 (74.70)	1.00	1.00
≥0.76	41 (50.62)	21 (25.30)	3.03 (1.57–5.85)	3.33 (1.64–6.77)
By quartiles (breaks/cell)[c]				
<0.36	8 (9.88)	20 (24.10)	1.00	1.00
0.36–0.49	12 (14.81)	20 (24.10)	1.51 (0.51–4.45)	1.92 (0.60–6.16)
0.50–0.76	20 (24.69)	22 (26.50)	2.27 (0.82–6.30)	3.33 (1.11–10.00)
≥0.77	41 (50.62)	21 (25.30)	4.88 (1.84–12.93)	7.15 (2.38–21.47)
Trend test			$P < 0.01$	$P < 0.01$

[a] Adjusted by age, sex, ethnicity, and smoking status (never, former and current).
[b] Dichotomized at the 75th percentile of breaks per cell in the controls.
[c] Categorized by quartiles of breaks per cell in the controls.

($P < 0.001$). Overall, BPDE sensitivity was associated with a significantly elevated risk for OPLs, with an OR of 12.96 (95% CI = 5.51–30.46) adjusted by age, sex, ethnicity, and smoking status. We also found a dose-response relationship between the number of breaks per cell and OPL risk. The risk increased with each stratum of increased breaks per cell; the adjusted ORs were 2.34 (95% CI = 0.21–26.2), 9.14 (95% CI = 1.01–82.58), and 54.04 (95% CI = 6.43–454.23) for the second, third, and fourth quartiles, respectively (Table 2).

Bleomycin sensitivity data were available for 81 cases and 83 controls. The results were similar to those from the BPDE sensitivity assay. A higher percentage of cases than controls were bleomycin sensitive (50.62 and 25.3%, respectively). The mean numbers of bleomycin-induced chromatid breaks per cell were 0.78 ± 0.37 for the cases and 0.57 ± 0.31 for the controls ($P < 0.001$). There was also a dose relationship with the degree of sensitivity. Individuals in the highest quartile (≥0.77 breaks/cell) were twice as likely than those in the third quartile and approximately four times as likely than those in the second quartile to have OPLs (Table 2).

There were no statistically significant differences in mutagen sensitivity by gender (Table 3). Furthermore, there were no significant associations between mean breaks per cell and smoking status, although the mean number of breaks per cell in subjects who had never smoked was consistently slightly higher than that in former or current smokers for both cases and controls and with both assays. However, age was associated with bleomycin sensitivity but not BPDE sensitivity in both the cases ($P = 0.004$) and the controls ($P = 0.003$). Older cases had a significantly higher number of bleomycin-induced breaks per cell than younger cases (0.91 and 0.67, respectively); similar results were seen for the controls (0.65 and 0.48, respectively).

We next performed stratified analysis to evaluate the joint effects of bleomycin and BPDE sensitivity (Table 4). Sensitivity to either mutagen was associated with significantly elevated risks with ORs of

Table 3 *Mean BPDE- and bleomycin-induced breaks per cell by host characteristics*[a]

	Cases				Controls			
Mutagen Variable	*n*	Mean	±SD	*P*	*n*	Mean	±SD	*P*
BPDE								
Overall[b]	71	1.05	0.40		71	0.55	0.27	
Sex								
Male	40	1.03	0.38		38	0.52	0.27	
Female	31	1.10	0.42	0.40	33	0.58	0.26	0.13
Age[c]								
<57 years	40	1.02	0.43		35	0.61	0.31	
≥57 years	31	1.11	0.35	0.27	36	0.49	0.21	0.09
Smoking status								
Never	12	1.17	0.26		8	0.69	0.28	
Former	33	1.05	0.41	0.20	41	0.54	0.27	0.08
Current	24	1.05	0.43	0.23	22	0.52	0.26	0.12
Bleomycin								
Overall[b]	81	0.78	0.37		83	0.57	0.31	
Sex								
Male	44	0.76	0.37		45	0.56	0.36	
Female	37	0.82	0.37	0.51	38	0.57	0.24	0.41
Age[c]								
<57 years	41	0.67	0.32		41	0.48	0.28	
≥57 years	40	0.91	0.38	0.004	42	0.65	0.32	0.003
Smoking status								
Never	16	0.84	0.42		10	0.66	0.46	
Former	37	0.82	0.38	0.92	48	0.57	0.32	0.81
Current	26	0.72	0.32	0.42	25	0.52	0.20	0.77

[a] Though the mean breaks per cell values were presented, the *P* was based on Wilcoxon's rank-sum test.
[b] $P < 0.001$ between cases and controls by Wilcoxon's rank-sum test.
[c] Dichotomized by median age of the controls.

Table 4 *Joint effects of bleomycin sensitivity and BPDE sensitivity*

Bleomycin sensitivity[a]	BPDE sensitivity[a]	Cases *n* (%)	Controls *n* (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[b]
Nonsensitive	Nonsensitive	9 (12.68)	43 (60.57)	1.00	1.00
Sensitive	Nonsensitive	7 (9.86)	10 (14.08)	3.34 (1.00–11.15)	8.85 (1.36–57.71)
Nonsensitive	Sensitive	27 (38.03)	11 (15.49)	11.73 (4.30–32.00)	12.49 (4.40–35.46)
Sensitive	Sensitive	28 (39.43)	7 (9.86)	19.11 (6.38–57.21)	49.91 (10.67–233.39)

[a] Sensitive, above the 75th percentile of breaks per cell in the controls; nonsensitive, <75th percentile.
[b] Adjusted by age, sex, ethnicity, and smoking status (former smokers and current smokers).

8.85 (95% CI = 1.36–57.71) for bleomycin and 12.49 (95% CI = 4.4–35.46) for BPDE. The risk associated with the sensitive phenotype for both assays was 49.91 (95% CI = 10.67–233.39), which was higher than that for either assay separately.

The cases exhibited significantly more aberrations at the 3p21.3 locus than controls did (51.13/1000 interphases *versus* 40.93/1000 interphases, respectively; $P < 0.001$; Table 5). In contrast, there was no significant difference in BPDE-induced chromosome aberrations at the 3q13 control locus, although cases did have slightly more aberrations than the controls did (9.7/1000 interphases *versus* 8.51/1000 interphases, respectively; $P = 0.06$).

We dichotomized the subjects by the 75th percentile of 3p21.3 aberrations in the controls, and those below this value were deemed nonsensitive. Sensitivity to induced 3p21.3 aberrations was associated with an adjusted 6-fold increased risk (OR = 6.08, 95% CI = 2.57–14.4; Table 5). Furthermore, when we categorized the subjects in quartiles of aberrations in controls, there was a significant dose-response relationship. These patterns were not evident for 3q13 aberrations.

DISCUSSION

Genetic instability, either spontaneous or mutagen induced, has been considered a predisposing factor for neoplastic transformation. In a cohort study of 3182 occupationally exposed workers, Hagmar *et al.* (35) reported that increased levels of chromosomal aberrations in lymphocytes was a significant predictor of subsequent cancer risk. This finding was confirmed in subsequent studies (36–38). A series of case control studies has demonstrated that mutagen sensitivity is an independent risk factor for tobacco-related cancers (7–21). In this study, we showed that subjects with OPLs also demonstrated greater sensitivity to bleomycin and BPDE than controls.

Hsu (7) suggested that the mutagen sensitivity assay indirectly measures the effectiveness of one or more DNA repair mechanisms. The following observations support this hypothesis. The relationship between chromosome instability syndromes and cancer susceptibility is well established. Specifically, the rates of spontaneous and bleomycin-, γ-radiation-, and UV light-induced chromosomal breakage are highest in patients with chromosome instability syndromes, such as Bloom syndrome, Fanconi's anemia, AT, Werner's syndrome, and XP. These patients also have defective DNA repair systems (9, 39–44). Furthermore, patients with AT, who are extremely sensitive to the clastogenic effects of X radiation and bleomycin, differ from healthy individuals in the speed with which aberrations induced by these agents are repaired but not in the number of aberrations produced (45, 46). Recently, studies have also shown that increased γ-radiation-induced DNA damage may be attributable to the disruption of genes that control the G_2 checkpoint (47). After DNA damage, these cells are unable to remain in the G_2 phase and progress into mitosis without sufficient time for DNA repair. In addition, a correlation between the cellular DNA repair capacity measured by the host cell reactivation assay and the frequency of mutagen-induced *in vitro* chromosomal breaks was reported (48).

Interestingly, as in lung (19) and upper aerodigestive tract cancers (15), bleomycin sensitivity and BPDE sensitivity had a joint effect on

Table 5 *BPDE-Induced chromosome aberrations at 3p21.3 locus in cases and controls*[a]

A.	Cases		Controls			
Locus	*n*	%	*n*	%	Crude OR (95% CI)	Adjusted OR (95% CI)[b]
3p21.3						
Nonsensitive[c]	19	33.93	42	71.19	1.00	1.00
Sensitive[c]	37	66.07	17	28.81		6.08 (2.57–14.40)
Quartiles of aberrations[d]						
0–35	2	3.57	13	22.03	1	1
36–38	1	1.79	13	22.03	0.50 (0.04–6.22)	0.62 (0.04–8.72)
39–45	16	28.57	16	27.12	6.50 (1.26–33.58)	6.67 (1.14–39.11)
≥46	37	66.07	17	28.82	14.15 (2.87–69.76)	17.77 (3.09–102.25)
3q13						
Nonsensitive[c]	33	61.11	44	74.58	1.00	1.00
Sensitive[c]	21	38.89	15	25.42	1.87 (0.84–4.16)	1.95 (0.84–4.53)
Quartiles of aberrations[d]						
0–5	6	11.11	5	8.47	1	1
6–7	6	11.11	18	30.51	0.28 (0.06–1.25)	0.19 (0.04–0.99)
8–10	21	38.89	21	35.59	0.83 (0.22–3.16)	0.89 (0.23–3.51)
≥11	21	38.89	15	25.43	1.17 (0.30–4.54)	1.16 (0.28–4.72)

B.	Mean no. of aberrations/1000 interphases				
	Cases		Controls		
Locus	*n*	Mean (±SD)	*n*	Mean (±SD)	*P*
3p21.3	56	51.13 ± 10.27	59	40.93 ± 10.03	<0.001
3q13	54	9.70 ± 3.49	59	8.51 ± 2.71	$P = 0.059$

[a] Though the mean number of aberrations was presented, the *P* was based on Wilcoxon's rank-sum test.
[b] Adjusted by age, sex, ethnicity, and smoking status.
[c] Sensitive, ≥75th percentile of aberrations per 1000 interphases in the controls; nonsensitive, <75th percentile.
[d] Aberrations per 1000 interphases.

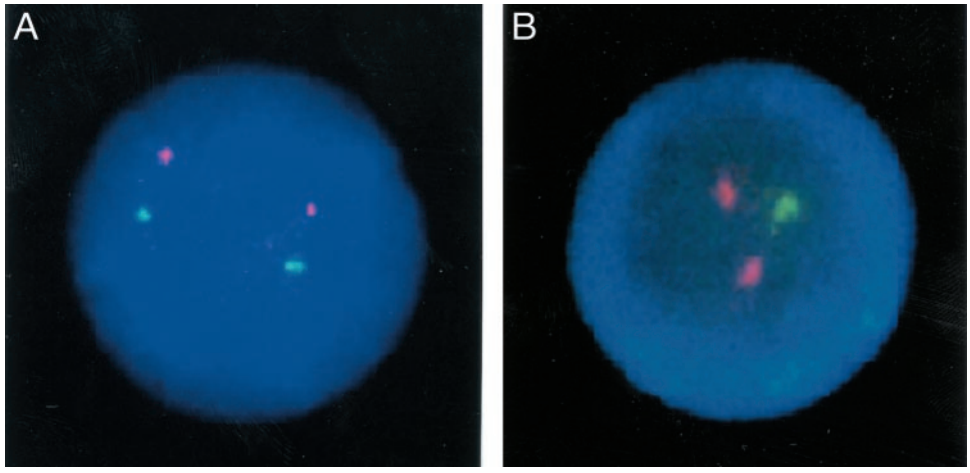

Fig. 1. Fluorescence *in situ* hybridization results from probes 3p21.3 labeled with spectrum *green* and 3q13 labeled with spectrum *orange*. The interphase on the *right* (*A*) represents a cell with normal copies of the chromosomal regions (two signals). The interphase on the *left* (*B*) represents a cell with 3p21.3 deletion (one *green* signal).

risk of OPLs. This suggests that multiple DNA repair defects may increase the risk of developing OPLs. Therefore, using multiple mutagens in parallel may help identify subgroups of individuals susceptible to carcinogens. Moreover, older subjects (>57 years) had more bleomycin-induced breaks than did the younger subjects, supporting the concept that older individuals have decreased DNA repair capacity. We did not find this association in our previous studies (15, 18, 19), perhaps because the age range of subjects was broader in this study.

It may be argued that the mutagen sensitivity of PBLs does not reflect mutagen sensitivity in the target tissue and that mutagen sensitivity may be a consequence rather than a cause of the increased cancer risk. However, in this study, no subjects had a cancer diagnosis. We and others (14, 16, 18, 49) have shown that the mutagen sensitivity profiles of both cancer cases and controls are not affected by tobacco use, alcohol use, or tumor stage. Denissenko *et al.* (50) found a similar adduct pattern in three different cell types: (*a*) HeLa cells; (*b*) bronchial cells; and (*c*) normal human fibroblasts, suggesting that the same adduct pattern is probably present in different target cells for oral tissue transformation. Seetharam *et al.* (51) reported that there were no significant differences in classes or types of mutations in the UV irradiation-treated plasmid replicated in XP lymphoblasts and XP fibroblasts. This suggests that the major features of mutagenesis in different cell types from the same individual may be similar. Udumudi *et al.* (52) also observed that among individuals with precancerous disease, genomic instability is observed not only in their cervical epithelial cells but also in their peripheral blood leukocytes. Therefore, the mutagen sensitivity of lymphocytes should reflect the repair capacity of the donor's target tissue. However, prospective studies of OPL patients carefully documenting outcome are warranted for validating this assay as a predictor of cancer susceptibility.

Whether BPDE exposure *in vitro* results in random or nonrandom genetic damages has not been evaluated definitively. Deletion of 3p is of particular interest as a molecular hot spot because it occurs early in oral premalignancies (53, 54). Furthermore, 3p is the most frequently reported chromosomal aberration in oral cancer (28, 29, 55). The deletion typically occurs in three discrete regions: (*a*) 3p14; (*b*) 3p21.2–21.3; and (*c*) 3p25 (28). Intact human chromosome 3 introduced into three different oral squamous cell carcinoma cell lines completely suppresses the tumorigenicity of each cell line (56). Transfer of DNA fragments from 3p21.3–21.2 into tumor cell lines suggests that the region has tumor suppressor activity (30, 31). Tumors associated with carcinogenic exposure may be especially susceptible to breakage at 3p. Sozzi *et al.* (32) reported that loss of heterozygosity of at least one locus of the *fragile histidine triad* gene at 3p was observed in 41 of 51 tumors in smokers (80%) but in only 9 of 40 tumors in nonsmokers (22%). Notably, analysis of accurate histories of smoking exposure in 6 of 8 nonsmoking patients with fragile histidine triad abnormalities revealed a significant exposure to passive smoke either at home or at work. Our data further support the hypothesis that 3p is a specific molecular target of BPDE, especially because we observed no significant differences at 3q13 that we selected as a control locus.

Possible limitations of this study is its small sample size. Nevertheless, the findings are provocative and warrant further investigation.

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