Benzo(*a*)pyrene Diol Epoxide-Induced Chromosome 9p21 Aberrations Are Associated with Increased Risk of Bladder Cancer

Jian Gu,[1] Yohei Horikawa,[1,3] Meng Chen,[1] Colin P. Dinney,[2] and Xifeng Wu[1]

Departments of [1]Epidemiology and [2]Urology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; and [3]Department of Urology, Akita University School of Medicine, Akita, Japan

Abstract

Purpose: **Loss of chromosome 9p21 is one of the most frequent genomic alterations in bladder cancer. Alterations of 9p21 and p16 are also frequently seen in the epithelial cells of chronic smokers. We hypothesize that 9p21 is a molecular target of benzo(*a*)pyrene diol epoxide (BPDE), the metabolic product of tobacco carcinogen benzo(*a*)pyrene, and 9p21 BPDE sensitivity is a genetic susceptibility factor for bladder cancer.**

Material and Methods: **In this case-control study of 203 bladder cancer cases and 198 matched healthy controls, we compared the frequencies of BPDE-induced 9p21 aberrations in cultured peripheral blood lymphocytes using fluorescent *in situ* hybridization and evaluated the association between 9p21 BPDE sensitivity and bladder cancer risk.**

Results: **We found that BPDE-induced chromosome 9p21 aberrations were significantly higher in peripheral blood lymphocytes of bladder cancer cases (20.76 ± 6.97 per 1,000) than those of controls (16.58 ± 7.07 per 1,000; $P < 0.0001$). However, no difference was observed for CEP9, a control centromere locus on chromosome 9. Using the median aberration value in the controls as a cutoff point to dichotomize BPDE sensitivity and after adjustment by age, sex, ethnicity, and smoking status, 9p21 BPDE sensitivity was associated with a significantly increased risk of bladder cancer (odds ratio, 5.29; 95% confidence interval, 3.26-8.59), whereas the odds ratio for the CEP9 locus was 0.99 (95% confidence interval, 0.66-1.50). There was also a dose-response relationship between the 9p21 BPDE sensitivity and increased risk for bladder cancer.**

Conclusion: **9p21 may be a molecular target for BPDE damage in bladder cancer cases and 9p21 BPDE sensitivity may be a marker of bladder cancer susceptibility.** (Cancer Epidemiol Biomarkers Prev 2008;17(9):2445–50)

Introduction

Bladder cancer is the fourth most common cancer in men and the ninth in women in the United States with an estimated 67,160 new cases and 13,750 deaths from this disease in 2007 (1). Previous epidemiologic evidence highlighted tobacco smoking as the predominant risk factor for bladder cancer. People who smoke are estimated to have 2- to 4-fold greater risk of developing bladder cancer than nonsmokers (2). Although there have been many studies of how tobacco carcinogens lead to genetic and molecular alterations, the molecular mechanisms of tobacco carcinogen caused pathogenesis of bladder are not fully understood.

Chromosome 9 is the most frequent genetic lesion in bladder carcinogenesis. Numerical cytogenetic studies have shown that chromosome 9p loss is an early event in bladder carcinogenesis because these alterations are observed in relatively early stage of bladder cancer and even in premalignant hyperplastic lesion (3-6). 9p21 region harbors *p16/CDKN2* and *p14*^{ARF} gene, which are involved in cell cycle regulation and cellular senescence, with p16 a critical component of the pRb pathway and p14^{ARF} a critical component of the p53 pathway. Dysfunction of these proteins is linked to tumorigenesis through hemizygous or homozygous deletions of 9p21 in a variety of cancers, including bladder cancer (7, 8). Previous molecular epidemiologic study showed a significant association between smoking and 9p21 alteration in lung cancer and its premalignant lesion, suggesting that 9p21 may be one of hotspots of tobacco carcinogen (9, 10).

Benzo(*a*)pyrene diol epoxide (BPDE) is the metabolic product of benzo(*a*)pyrene, a major constituent of tobacco smoke, and has been tested in numerous epidemiologic studies examining mutagen sensitivity (11). In previous studies of tobacco-related cancers, we found that BPDE-induced chromosomal breaks occur more frequently in peripheral blood lymphocytes (PBL) from cases than in those from healthy controls and that mutagen-induced chromosomal breaks are not random but apparently reflect inherited genetic susceptibility of specific loci to damage by carcinogens (12, 13). Therefore, measuring these specific chromosome aberrations may have the potential to predict an individual's genetic susceptibility to tobacco-related cancer. In an earlier pilot case control study of bladder cancer, we tested 9p21 sensitivity to BPDE using locus specific fluorescence *in situ* hybridization (FISH) technique and revealed that

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Requests for reprints: Jian Gu, Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center, Unit 1340, 1155 Pressler Boulevard, Houston, TX 77030. Phone: 713-792-8016; Fax: 713-792-4657. E-mail: jiangu@mdanderson.org

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9p21 may be a hotspot for BPDE and 9p21 sensitivity to BPDE may be associated with an increased risk of bladder cancer (14). In this current report, we tripled the sample size and validated previous findings from the pilot study (14) that BPDE-induced 9p21 aberration is associated with an increased risk of bladder cancer.

Materials and Methods

Study Subjects. Our ongoing hospital-based bladder cancer case-control study was conducted at The University of Texas M.D. Anderson Cancer Center, Houston, Texas. The cases were recruited for a molecular epidemiologic study of susceptibility markers for bladder cancer at the hospital beginning in July 1999. The inclusion criteria were diagnosis within 1 year of recruitment, no previous chemotherapy or radiotherapy, and histologic confirmation. There were no age, gender, ethnicity, and cancer stage restrictions. Each new patient was approached; those interested in participating were asked to sign a consent form and to complete two structured questionnaires. The control subjects had no history of cancer (except nonmelanoma skin cancer) and were selected from the Kelsey-Seybold Foundation, the largest multispecialty physician group in the greater Houston area, Texas. The potential controls were identified by reviewing short survey forms distributed to individuals coming to the clinic. Most controls came to the clinic for annual health check-ups. The potential control subjects were subsequently contacted by telephone to confirm their willingness to participate, and an appointment was scheduled at a Kelsey Seybold clinic site convenient to the participant.

On the day of the interview, the controls visited the clinic specifically for the purpose of participating in our study but not for any treatment purposes. If the person refused to participate or was deemed ineligible, another potential control was selected. Upon case recruitment, we randomly identified a control matched to the case based on gender, age (±5 years), and ethnicity. The response rates for the ongoing case control study were 92% for cases and 76.7% for controls. For this study, the cases were selected consecutively from the parent case control study during the years from 2003 to 2005. The controls were matched to the cases on age, gender, and ethnicity and selected from controls enrolled during the same period.

Collection of Epidemiologic Data. After signing the informed consent form, study participants completed a 30-min questionnaire administered by trained interviewers. The questionnaire elicited information about demographics, smoking history, family history of cancer, and medical history. When the interview was completed, blood was drawn into sodium heparinized tubes for cytogenetic and molecular analyses.

BPDE Sensitivity Assay. FISH was used to measure BPDE-induced 9p21 aberration as previously described (14). Briefly, whole blood (1 mL) was cultured in 9 mL of RPMI 1640 (JRM Biosciences) with 20% FCS and 1.25% phytohemagglutinin (Wellcome Research Laboratories) at 37°C for 72 h. BPDE was added at the final concentration of 2 μmol/L for 24 h. After routine blocking with colcemid, hypotonic treatment, and fixation, cell suspensions were stored at −20°C until processing for FISH experiments. The 9p21 aberrations were detected by FISH with an LSI 9p21/CEP9 dual-color probe (Vysis), which is a mixture of the locus-specific identifier 9p21 probe, labeled with SpectrumOrange, and the centromere enumeration probe for chromosome 9 probe, labeled with SpectrumGreen. The 9p21 SpectrumOrange probe spans ~190 kb and contains a number of genetic loci, including the microsatellite markers *D9S1749*, *D9S1747*, *P16INK4A*, *P14ARF*, *D9S1748*, *P15INK4B*, and *D9S1752.* The CEP9 SpectrumGreen probe hybridizes to alpha satellite sequences specific to chromosome 9. CEP9 was selected as the control locus because it is not deleted in bladder cancer.

The CEP9 probe also served as a control for differences in chromatin structure (which may affect hybridization) between 9p21 and the rest of the genome. Dual-color FISH was done with LSI hybridization buffer (Vysis) according to the manufacturer's instructions. The cells were counterstained with DAPI and viewed through a fluorescence microscope (Olympus). The criteria for scoring FISH signals were as follows: the nuclei should not overlap; signal intensity should be uniform; minor hybridization spots smaller and less intense than real signals were excluded; signals should be completely separated from each other; and paired or close signals were counted as one signal. The most common abnormal event was deletion. All the assays in this study were done by the same individual who was blinded to the case control status. We counted 1,000 interphase cells for each sample to detect and enumerate chromosomal aberrations. We do not have repeat samples from the same individuals at different time points. To show the reproducibility of 9p21 deletion assay, we did two independent experiments starting from two different aliquots obtained at the same time for 10 control samples. The mean intra-individual coefficient of variation (CV) was 10%, which was significantly smaller than the inter-individual CV (21%). The mean intra-assay and inter-assay CV was both 8%.

Statistical Analysis. BPDE sensitivity was analyzed both as a continuous variable and as a categorical variable. The χ^2 test was used to test for differences in distribution between the cases and controls for gender, ethnicity, and smoking status. The Student's t test was conducted to compare the differences between cases and controls for continuous variables, including age, pack-years, and BPDE sensitivity. The BPDE sensitivity was also analyzed as a categorical variable by dichotomizing the subjects at the median value of BPDE-induced 9p21 aberrations in controls, or by grouping the subjects according to the tertile distribution of BPDE-induced 9p21 aberrations in controls. To control the confounding, unconditional multiple logistic regression analysis was conducted and adjusted odds ratios (OR) along with 95% confidence intervals (95% CI) were calculated by the STATA software (Stata Corp.). All statistical tests were two sided.

Results

This study consisted of 203 patients with bladder cancer and 198 control subjects (Table 1). There were no significant differences between cases and controls in

Table 1. Distribution of selected variables by cases and controls

Variables	Cases (n = 203)	Controls (n = 198)	P
Age			
Mean (SD)	63.34 (11.78)	62.64 (11.40)	0.727
Ethnicity			
Caucasian	182 (90.10)	176 (88.89)	
African-American	12 (5.94)	13 (6.57)	
Mexican-American	8 (3.96)	8 (4.04)	
Others	0 (0.00)	1 (0.51)	0.777
Gender			
Male	156 (76.85)	151 (76.26)	
Female	47 (23.15)	47 (23.74)	0.89
Smoking status			
Never	56 (27.59)	78 (39.39)	
Former	95 (46.80)	99 (50.00)	
Current	52 (25.62)	21 (10.61)	<0.001
Ever	147 (72.41)	120 (60.61)	0.012
Pack-year*			
Mean (SD)	42.60 (32.49)	29.90 (30.84)	<0.001
9p21 aberrations			
Mean (SD)	20.76 (6.97)	16.58 (7.07)	<0.0001
CEP9 aberrations			
Mean (SD)	17.52 (7.28)	17.82 (7.33)	0.689

*Smokers only.

terms of age, gender, and ethnicity. The majority of the subjects were Caucasian (90.1% for cases and 88.9% for controls). Cases had a significantly higher percentage of current (25.62%) smokers than the controls (10.61%; $P < 0.001$). Cases had a heavier smoking history than controls (mean pack-years: 42.60 versus 29.90; $P < 0.001$). Cases exhibited significantly more BPDE-induced aberrations per 1,000 interphase cells than controls at the 9p21 locus (20.76 $\pm$ 6.97 versus 16.58 $\pm$ 7.07, $P < 0.0001$), but not at the CEP9 locus (17.52 $\pm$ 7.28 versus 17.82 $\pm$ 7.33, $P = 0.689$; Table 1). None of the host characteristics (age, gender, and smoking status) and tumor characteristics (stage and grade) exhibited significant effect on BPDE-induced 9p21 deletions (Table 2). Stage T_4 patients seemed to have the highest 9p21 deletions, but the number of cases is too small and the difference did not reach statistical significance (T_4 versus T_0, 26.50 $\pm$ 14.1 versus 21.18 $\pm$ 7.34, $P = 0.14$).

We then performed unconditional logistic regression analysis to assess the association between BPDE sensitivity and risk of bladder cancer. We used the median value of the BPDE-induced 9p21 aberrations in controls as the cutoff point to dichotomize the subjects into nonsensitive and sensitive groups (Table 3). After adjusting for age, gender, ethnicity, and smoking status, individuals with high BPDE sensitivity were associated with a significantly increased bladder cancer risk (OR, 5.28; 95% CI, 3.26-8.59). The OR for BPDE sensitivity at CEP9 was 0.99 (95% CI, 0.66-1.50). In stratified analysis, it seems that BPDE high sensitivity at 9p21 was associated with the highest bladder cancer risk in the oldest age group (≥70 years old; OR, 11.23; 95% CI, 3.78, 33.38; Table 3).

We further categorized subjects into tertiles of the BPDE sensitivity, based on its distribution in the controls. A dose response was observed between the BPDE sensitivity at 9p21 and bladder cancer risk (Table 4). Compared with individuals with the lowest tertile of the BPDE sensitivity, individuals with the 2nd and the highest tertiles of BPDE sensitivity exhibited significantly elevated risks of bladder cancer with adjusted ORs of 13.66 (95% CI, 4.66-40.05) and 23.2 (95% CI, 7.98-67.44), respectively (P for trend < 0.001). In contrast, the ORs for BPDE sensitivity at CEP9 in the 2nd and 3rd tertiles were 1.07 (95% CI, 0.64-1.80) and 0.87 (95% CI, 0.53-1.43), respectively (Table 4).

Discussion

In this study, updating our previous pilot study using much larger sample size, we found that high

Table 2. Mean numbers of 9p21 aberrations by host characteristics in cases and controls

	Cases			Controls		
	n	Mean number (SD) of 9p21 aberrations	P	n	Mean number (SD) of 9p21 aberrations	P
Gender						
Male	156	21.03 (7.36)		151	16.51 (6.73)	
Female	47	19.89 (5.43)	0.33	47	16.81 (8.14)	0.80
Age						
<62	75	21.28 (7.56)		75	16.33 (6.47)	
≥62	128	20.46 (6.61)	0.42	123	16.73 (7.43)	0.70
Smoking status						
Never	56	21.30 (7.10)		78	17.42 (8.02)	
Former	95	20.35 (7.16)		99	15.90 (6.35)	
Current	52	20.94 (6.53)	0.74	21	16.67 (6.50)	0.08
Ever	147	20.56 (6.93)	0.50	120	16.03 (6.35)	0.18
Stage						
T_0 (T_a + T_{is})	60	21.18 (7.34)				
T_1	59	19.96 (5.42)				
T_2	53	20.92 (7.04)				
T_3	11	19.18 (3.25)				
T_4	8	26.50 (14.1)	0.82			
Grade						
1	7	24.57 (8.79)				
2	54	21.63 (6.97)				
3	129	20.40 (6.86)	0.12			

Table 3. BPDE-induced 9p21 deletions and bladder cancer risk

Variables	*n* (%)		Adjusted OR (95% CI)*
	Cases	Controls	
Overall			
Low sensitivity †	34 (16.7)	98 (49.5)	
High sensitivity	169 (83.3)	100 (50.5)	5.28 (3.26-8.59)
Age			
Dichotomize			
<62			
Low sensitivity	13 (17.3)	36 (48.0)	
High sensitivity	62 (82.7)	39 (52.0)	4.93 (2.23-10.90)
≥62			
Low sensitivity	21 (16.4)	62 (50.4)	
High sensitivity	107 (83.6)	61 (49.6)	5.49 (2.94-10.27)
Tertile analysis			
≤60			
Low sensitivity	11 (16.2)	35 (51.5)	
High sensitivity	57 (83.8)	33 (48.5)	6.36 (2.71-14.91)
61-69			
Low sensitivity	16 (23.5)	35 (46.7)	
High sensitivity	52 (76.5)	40 (53.3)	2.89 (1.34-6.24)
≥70			
Low sensitivity	7 (10.4)	28 (50.9)	
High sensitivity	60 (89.6)	27 (49.1)	11.24 (3.78-33.38)
Gender			
Male			
Low sensitivity	27 (17.3)	75 (49.7)	
High sensitivity	129 (82.7)	76 (50.3)	5.37 (3.07-9.37)
Female			
Low sensitivity	7 (14.9)	23 (48.9)	
High sensitivity	40 (85.1)	24 (51.1)	5.56 (2.03-15.25)
Smoking status			
Never			
Low sensitivity	5 (8.9)	31 (39.7)	
High sensitivity	51 (91.1)	47 (60.3)	7.13 (2.52-20.20)
Former			
Low sensitivity	19 (20.0)	56 (56.6)	
High sensitivity	76 (80.0)	43 (43.4)	5.19 (2.72-9.90)
Current			
Low sensitivity	10 (19.2)	11 (52.4)	
High sensitivity	42 (80.8)	10 (47.6)	4.20 (1.28-13.84)

*Adjusted by age, gender, ethnicity, and smoking status.
†Dichotomized at the median value of 9p21aberrations in the controls.

9p21 sensitivity to BPDE induction exhibited a 5-fold significantly increased risk of bladder cancer with a clear dose-response relationship. Furthermore, the larger sample size allowed us to perform stratified analysis that suggested that smoking status may modify the risk associated with high BPDE 9p21 sensitivity.

There is considerable inter-individual variation in sensitivity to environmental mutagens. This variation may be measured by mutagen sensitivity assay. The classic mutagen sensitivity assay, measured by quantifying the chromatid breaks induced by mutagens in short-term cultures of PBLs, has been used as an indirect measure of DNA repair capacity (15). Hsu et al. (15) hypothesized that in response to mutagen exposure, higher levels of genetic damage would accumulate in people with suboptimal DNA repair than in normal individuals. Therefore, the level of chromatid breaks induced by a mutagen challenge would reflect an individual's ability to repair DNA damage. Numerous case-controls studies and prospective studies have shown that high mutagen-induced chromatid breaks in short-term cultured PBLs is a significant independent cancer risk predictor for all the major types of cancer (11). In this study, we went one further step by measuring locus-specific chromosome alterations induced by mutagens rather than counting gross chromatid breaks. There is evidence that chromosome instability is not randomly distributed but located at specific points in the genome (16-21). Highly recombinogenic regions and regions prone to breakage in humans were shown to be the nonrandom targets of mutagen action (22). Such fragile sites can be induced by treatment with mutagens and correlate with two thirds of the recurrent chromosomal break points in cancer where most proto-oncogenes, growth factor genes, and tumor suppressor genes are often located (22, 23). Specific chromosome aberrations have been observed in patients with many types of tumors. In bladder cancer, many previous studies have shown that 9p21 aberration is the most frequent event in bladder tumors.

We used PBLs as a surrogate tissue in this study. Ideally, normal target tissue (e.g., epithelial cells) should be used as the experimental material in studies of the early events in carcinogenesis. However, because it is difficult to obtain large samples of cultured epithelial cells, lymphocytes may be used initially as a surrogate tissue. For example, nonrandom cytogenetic anomalies of chromosome 13 in retinoblastoma and chromosome 11 in Wilms' tumor were first found in the lymphocyte cultures of children who also had physical birth defects and mental retardation (16-19). Later, it became apparent that similar specific types of anomalies were present in the tumor cells of children with retinoblastoma and Wilms' tumor. In individuals with cervical precancerous disease, genomic instability occurs in both cervical epithelial cells and in PBLs (24). Concordant aberrations of specific chromosome regions have been observed in lymphocytes and other tumor tissues (25-28). These findings suggest that the genetic instability in different cell types from the same individual may be similar. Egeli et al. (21) found that spontaneous 3p14 gaps or breaks in PBLs were most frequent in lung cancer patients, less frequent in the relatives of lung cancer patients, and least frequent in healthy controls. In addition, several previous studies have shown that mutagen-induced chromosome damages in PBLs have high heritability (29). This current study strongly suggests that 9p21 is a target region of mutagen in bladder carcinogenesis.

Cigarette smoke contains a mixture of highly mutagenic compounds, including aromatic amines (e.g.,

Table 4. Dose-response association between BPDE-induced 9p21 aberration and bladder cancer risk by tertile analysis

	n (%)		Adjusted OR (95% CI)*
	Cases	Controls	
9p21			
1st Tertile	4 (2.0)	55 (27.8)	Reference
2nd Tertile	76 (37.4)	72 (36.4)	13.66 (4.66-40.05)
3rd Tertile	123 (60.6)	71 (35.9)	23.20 (7.98-67.44)
CEP9			
1st Tertile	68 (33.8)	60 (30.6)	Reference
2nd Tertile	64 (31.8)	64 (32.7)	1.07 (0.64-1.80)
3rd Tertile	69 (34.3)	72 (36.7)	0.87 (0.53-1.43)

*Adjusted by age, gender, ethnicity, and smoking status.

4-ABP) and polycyclic aromatic hydrocarbons [e.g., benzo(*a*)pyrene]. Aromatic amines are considered the key etiologic component of tobacco smoke that causes bladder cancer. There is less certainty that benzo(*a*)pyrene or other polycyclic aromatic hydrocarbons cause bladder cancer. Most of these carcinogens form bulky DNA adducts that require nucleotide excision repair. We used BPDE-induced 9p21 aberration as a representative marker for bladder cancer susceptibility; however, other tobacco carcinogens may also cause alterations at this target site. Previous studies have used BPDE as a challenging mutagen and demonstrated that deficiency in repairing BPDE induced DNA damage pathway was potential risk factors for a number of cancers, including bladder cancer (11, 30-32). This current study suggests that the deficiency in repair bulky DNA adducts may cause increased 9p21 aberrations in the PBLs of susceptible individuals.

We did not measure the baseline 9p21 deletion in this current study. In a previous small study (33), we measured the baseline chromosome 9 aberrations using whole chromosome painting technique (WCP). We further performed 9p21-specific FISH (using the same 9p21/CEP9 dual-color probe as this current study) on slides from 10 representative cases with the highest frequency of 9p deletions and from 10 controls with the lowest frequency of 9p deletions, as determined by WCP. We found that the baseline 9p21 aberrations occurred at a lower frequency (cases versus controls, 12.1 versus 6.4 per 1,000 interphase cells, $P < 0.05$). The baseline 9p21 deletion exists at lower frequency than BPDE induced 9p21 deletion as shown in this current study. Some of these baseline alterations in 9p21 may be related to chronic exposure of carcinogens, but not related to BPDE challenge used in our *in vitro* assay and the BPDE-induced 9p21 deletions may reflect a constitutional host susceptibility to DNA damage. We did not find a significant association between BPDE-induced 9p21 deletion and the level of tobacco smoking in this study. Theoretically, tobacco smoking may increase baseline chromosome aberrations in PBLs due to chronic exposure to mutagens. However, in his study, we measured the BPDE-induced 9p21 deletion, which we believe is more a reflection of a constitutional host susceptibility to DNA damage than a marker of environmental exposure. The acute increase of 9p21 deletions upon BPDE challenge may mask the modest chronic effect of tobacco smoking on baseline deletions. Even in the case of baseline chromosome breaks in PBLs, the data of smoking effect have been inconsistent (34-37). The association between smoking and baseline or mutagen-induced 9p21 breaks warrants further investigation.

This is a retrospective case-control study and we do not have repeated measurement of the same individual at different times. Several previous studies have shown the repeatability of *in vitro* mutagen-induced chromosome breaks in PBLs over a relatively long time period. Cloos et al. (38) measured mutagen sensitivity of multiple samples from the same person at various time intervals (1-12 weeks) in a total of nine individuals and found that the mean inter-individual variation (0.35 breaks per cell) greatly exceeded the mean intra-individual variation (0.08 breaks per cell). In another clinical trial to evaluate the effect of vitamin C supplementation on *in vitro* mutagen sensitivity, King et al. (39) assessed mutagen-induced chromosome breaks in PBLs at baseline and at weeks 4, 16, and 20 and found that the mutagen sensitivity level was relatively stable over the 20-week period.

Shigyo et al. (40) examined urine samples for follow-up after transurethral resection and showed that chromosome 9 alterations could predict the recurrence of bladder cancer. It is interesting to investigate whether BPDE-induced 9p21 aberrations could serve as a prognostic marker for superficial bladder cancer recurrence. Because 70% to 80% of bladder cancer cases are superficial cases, and recurrence is a hallmark of superficial cases with a frequency of about 70%, the ability to predict recurrence is clinically very important. A large study with sufficient follow-up data is needed to address this interesting question. In addition, the biological mechanism by with BPDE induces more 9p21 aberrations in susceptible individuals and genes involved in the process warrant further studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Jemal A, Siegel R, Ward E, Murray T, XU J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
2. Vineis P, Alavanja M, Buffler P, et al. Tobacco and Cancer: Recent Epidemiological Evidence. J Natl Cancer Inst 2004;96:99–106.
3. Sugano K, Kakizoe T. Genetic alterations in bladder cancer and their clinical applications in molecular tumor staging. Nat Clin Pract Urol 2006;41:642–52.
4. Stadler WM, Steinberg G, Yang X, Hagos F, Turner C, Olopade OI. Alterations of the 9p21 and 9q33 chromosomal bands in clinical bladder cancer specimens by fluorescence *in situ* hybridization. Clin Cancer Res 2001;7:1676–82.
5. Hartmann A, Moser K, Kriegmair M, Hofstetter A, Hofstaedter F, Knuechel R. Frequent genetic alterations in simple urothelial hyperplasias of the bladder in patients with papillary urothelial carcinoma. Am J Pathol 1999;154:721–7.
6. van Oers JM, Adam C, Denzinger S, et al. Chromosome 9 deletions are more frequent than FGFR3 mutations in flat urothelial hyperplasia of the bladder. Int J Cancer 2006;119:1212–5.
7. Carins P, Tokino K, Eby Y, Sidransky D. Homozygous deletions of 9p21 in primary human bladder tumors detected by comparative multiplex polymerase chain reaction. Cancer Res 1994;54:1422–4.
8. Carins P, Polascik TJ, Eby Y, Tokino K, Califano J, Merlo A. Frequency of homozygous deletion at p16/CDKN2 in primary human tumors. Nature Genet 1995;11:210–2.
9. Mao L, Lee JS, Kurie JM, et al. Clonal genetic alterations in the lungs of current and former smokers. J Natl Cancer Inst 1997;89:857–62.
10. Wistuba II, Lam S, Behrens C, et al. Molecular damage in the bronchial epithelium of current and former smokers. J Natl Cancer Inst 1997;89:1366–72.
11. Wu X, Gu J, Spitz MR. Mutagen sensitivity: a genetic predisposing factor for cancer. Cancer Res 2007;67:3493–5.
12. Wu X, Zhao Y, Honn SE, Tomlinson GE, Minnua JD, Hong WK. Benzo(*a*)pyrene diol epoxide-induced 3p21.3 aberrations and genetic predisposition to lung cancer. Cancer Res 1998;58:1605–8.
13. Wu X, Lippman SM, Lee JJ, Zhu Y, Wei QV, Thomas M. Chromosome instability in lymphocytes: A potential indicator of predisposition to oral premalignant lesions. Cancer Res 2002;62:2813–8.
14. Hazra A, Grossman HB, Zhu Y, Luo S, Spitz MR, Wu X. Benzo(*a*)pyrene diol epoxide-induced 9p21 aberrations associated

with genetic predisposition to bladder cancer. Genes Chromosomes Cancer 2004;41:330–8.
15. Hsu TC, Johnston DA, Cherry LM, et al. Sensitivity to genotoxic effects of bleomycin in humans: possible relationship to environmental carcinogenesis. Int J Cancer 1989;43:403–9.
16. Yunis JJ, Ramsay N. Retinoblastoma and subband deletion of chromosome 13. Am J Dis Child 1978;132:161–3.
17. Yunis JJ, Ramsay N. Familial occurrence of the aniridia Wilm's tumor syndrome with deletion 11p13–14.1. J Pediatr 1980;96:1027–30.
18. Lee WH, Bookstein R, Hong F, Young LJ, Shew JY, Lee EY. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. Science 1987;235:1394–9.
19. Call KM, Glaser T, Ito CY, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell 1990;60:509–20.
20. Sundareshan TS, Augustus M. Expression of common fragile sites in lymphocytes of Wilms tumor in patients, their parents, and siblings: a study of ten families. Cancer Genet Cytogenet 1995;84:51–5.
21. Egeli U, Karadag MK, Tunca B, Ozyardimci N. The expression of common fragile sites and genetic predisposition to squamous cell lung cancers. Cancer Genet Cytogenet 1987;95:153–8.
22. Yunis JJ, Soreng AL, Bowe AE. Fragile sites are targets of diverse mutagens and carcinogens. Oncogene 1987;1:59–69.
23. Yunis JJ. Multiple recurrent genomic rearrangements and fragile sites in human cancer. Somat Cell Mol Genet 1987;13:397–403.
24. Udumudi A, Jaiswal M, Rajeswari N, et al. Risk assessment in cervical dysplasia patients by single cell gel electrophoresis assay: a study of DNA damage and repair. Mutat Res 1998;412:195–205.
25. Pathak S, Hopwood VL, Hughes JI, Jackson GL. Identification of colon cancer-predisposed individuals: a cytogenetic analysis. Am J Gastroenterol 1991;86:679–84.
26. Bonassi S, Abbondandolo A, Camurri L, et al. Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? Preliminary results of an Italian cohort study. Cancer Genet Cytogenet 1995;79:133–5.
27. Hagmar L, Brogger A, Hansteen I, et al. Cancer risk in humans predicted by increased levels of chromosome aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res 1994;54:2919–22.
28. Bonassi S, Hagmar L, Stromberg U, et al. Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. European Study Group on Cytogenetic Biomarkers and Health. Cancer Res 2000;60:1619–25.
29. Bonassi S, Znaor A, Norppa H, Hagmar L. Chromosomal aberrations and risk of cancer in humans: an epidemiologic perspective. Cytogenet Genome Res 2004;104:376–82.
30. Wu X, Gu J, Amos CI, Jiang H, Hong WK, Spitz MR. A parallel study of *in vitro* sensitivity to benzo[*a*]pyrene diol epoxide and bleomycin in lung carcinoma cases and controls. Cancer 1998;83:1118–27.
31. Schabath MB, Spitz MR, Grossman HB, et al. Genetic instability in bladder cancer assessed by the comet assay. J Natl Cancer Inst 2003; 95:540–7.
32. Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. J Natl Cancer Inst 2000; 92:874–97.
33. Shao L, Lerner SL, Bondaruk J, et al. Specific chromosome aberrations in peripheral blood lymphocytes are associated with risk of bladder cancer. Genes Chromosomes Cancer 2004;41:379–89.
34. Kasuba V, Sentija K, Garaj-Vrhovac V, Fuciæ A. Chromosome aberrations in peripheral blood lymphocytes from control individuals. Mutat Res 1995;346:187–93.
35. Galloway SM, Berry PK, Nichols WW, et al. Chromosome aberrations in individuals occupationally exposed to ethylene oxide, and in a large control population. Mutat Res 1986;170:55–74.
36. Maffei F, Angelini S, Forti GC, et al. Spectrum of chromosomal aberrations in peripheral lymphocytes of hospital workers occupationally exposed to low doses of ionizing radiation. Mutat Res 2004; 547:91–9.
37. Milillo CP, Gemignani F, Sbrana I, Carrozzi L, Viegi G, Barale R. Chromosome aberrations in humans in relation to site of residence. Mutat Res 1996;360:173–9.
38. Cloos J, Steen I, Joenje H, et al. Association between bleomycin genotoxicity and non-constitutional risk factors for head and neck cancer. Cancer Lett 1993;74:161–5.
39. King TM, Trizna Z, Wu X, et al. A clinical trial to evaluate the effect of vitamin C supplementation on *in vitro* mutagen sensitivity. The University of Texas M. D. Anderson Clinical Community Oncology Program Network. Cancer Epidemiol Biomarkers Prev 1997;6:537–42.
40. Shigyo M, Sugano K, Tobisu K, Tsukamoto T, Sekiya T, Kakizoe T. Molecular follow-up of newly diagnosed bladder cancer using urine samples. J Urol 2000;166:1280–5.

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