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Less Efficient G_2-M Checkpoint Is Associated with an Increased Risk of Lung Cancer in African Americans

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Abstract

Cell cycle checkpoints play critical roles in the maintenance of genomic integrity. The inactivation of checkpoint genes by genetic and epigenetic mechanisms is frequent in all cancer types, as a less-efficient cell cycle control can lead to genetic instability and tumorigenesis. In an on-going case-control study consisting of 216 patients with non–small cell lung cancer, 226 population-based controls, and 114 hospital-based controls, we investigated the relationship of γ-radiation-induced G_2-M arrest and lung cancer risk. Peripheral blood lymphocytes were cultured for 90 hours, exposed to 1.0 Gy γ-radiation, and harvested at 3 hours after γ-radiation treatment. γ-Radiation-induced G_2-M arrest was measured as the percentage of mitotic cells in untreated cultures minus the percentage of mitotic cells in γ-radiation-treated cultures from the same subject. The mean percentage of γ-radiation-induced G_2-M arrest was significantly lower in cases than in population controls (1.18 versus 1.44, $P < 0.01$) and hospital controls (1.18 versus 1.40, $P = 0.01$). When dichotomized at the 50th percentile value in combined controls (population and hospital controls), a lower level of γ-radiation-induced G_2-M arrest was associated with an increased risk of lung cancer among African Americans after adjusting for baseline mitotic index, age, gender, and pack-years of smoking [adjusted odd ratio (OR), 2.25; 95% confidence interval (95% CI), 0.97–5.20]. A significant trend of an increased risk of lung cancer with a decreased level of G_2-M arrest was observed ($P_{trend} = 0.02$) among African Americans, with a lowest-versus-highest quartile adjusted OR of 3.74 (95% CI, 0.98–14.3). This trend was most apparent among African American females ($P_{trend} < 0.01$), with a lowest-versus-highest quartile adjusted OR of 11.75 (95% CI, 1.47–94.04). The results suggest that a less-efficient DNA damage–induced G_2-M checkpoint is associated with an increased risk of lung cancer among African Americans. Interestingly, we observed a stronger association of DNA damage–induced G_2-M arrest and lung cancer among African Americans when compared with Caucasians. If replicated, these results may provide clues to the exceedingly high lung cancer incidence experienced by African Americans.

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Introduction

Genomic integrity of mammalian cells is maintained by a complex, highly preserved, and well-regulated defense system consisting of DNA repair, cell cycle checkpoints, and apoptosis. Cell cycle checkpoints determine a temporary arrest at a specific stage of the cell cycle to allow the cell to correct possible defects (1,2). At least two checkpoints detect DNA damage: one at the G_1-S transition and one at the G_2-M transition. The G_1-S checkpoint prevents the cell from replicating damaged DNA. Considerable experimental evidence support the view that the loss of the G_1-S checkpoint can lead to genomic instability and inappropriate survival of genetically damaged cells and contribute to the evolution of cells to malignancy (3–7). The G_2-M checkpoint is activated by DNA damage and by incompletely replicated DNA. This checkpoint prevents chromosome segregation if the chromosome is not intact. The signaling pathway, leading to G_2 arrest after DNA damage, is frequently altered or mutated in human cancer (8–10).

DNA lesions may be left unrepaired in cells with disrupted or suboptimal cell cycle checkpoints. If unrepaired DNA lesions are replicated or segregated, the genomic integrity of the progeny cells will be compromised. Several observations suggest that defects in the regulation of these transitions may play a critical role in human tumorigenesis. For example, nonneoplastic cells from individuals with familial cancer predisposition display a higher than average frequency of mitotic chromosomal breaks after irradiation (11,12). Cells from patients with ataxia telangiectasia undergo “suboptimal arrest” after irradiation in the G_2 phase (13–16). Altered expressions of cyclins A, B, and CDC2, which are all potential targets of mitotic checkpoint control, occur in some cancers (17). Patients with cancers of the head and neck, and lung, have been observed to express a significantly higher frequency of carcinogen-induced chromosome breaks than cancer-free control subjects (18–21). Mutations in cell cycle control genes, such as *p53* and *p21*, have also been directly linked to chromosomal aberrations and genomic instability (22–24). More recently, Wu et al. reported that lower γ-radiation-induced accumulations of cells in the S and G_2 phases were associated with an increased risk of lung cancer (25).

Lung cancer is the leading cause of cancer-related deaths in the United States. Cigarette smoking is the number one risk factor for lung cancer and is responsible for >80% of the lung cancer burden (26,27). However, the fact that only 10% of smokers develop lung cancer suggests that genetic and acquired host factors modulate susceptibility to tobacco carcinogens (28). We hypothesize that individuals with a less-efficient DNA damage–induced G_2-M cell cycle checkpoint have an increased risk of lung cancer. In an on-going case-control study of lung cancer, we investigated the associations of γ-radiation-induced G_2-M arrest and lung cancer risk.

Materials and Methods

Study population

The study population accrual and eligibility criteria have been described previously (29). The 216 lung cancer patients were recruited from seven hospitals in the Metropolitan Baltimore area. All cases were histologically confirmed non–small cell primary lung tumors. Population controls (n = 226) were recruited from the same Maryland counties of residence as the lung cancer cases by screening information obtained from the Department of Motor Vehicles, which allowed us to obtain a random sample of controls frequency-matched to the cases by gender, race, and age. Hospital controls (n = 114) were cancer-free patients recruited from the same hospital as cases and were frequency matched to the cases by gender, race, age, and smoking status. The overall participation rates of the study population as of May 2004 are the following: (a) cases: among 3,924 potential lung cancer patients screened, 3,701 completed eligibility

screening, 579 were eligible, and 522 (90%) participated in the study; (b) population controls: among 2,618 screened, 921 completed eligibility screening, 418 were eligible, and 369 (88%) participated in the study; (c) hospital controls: among 1,596 screened, 1,454 completed eligibility screening, 334 were eligible, and 293 (88%) participated in the study. Among the cases, the distribution of gender and race was similar between responders and nonresponders, and among the control groups, the distribution of gender was also similar. However, African American males were significantly more likely to be the nonresponders in both population and hospital control groups.

Eligibility criteria

Eligible subjects had to be either Caucasian or African American free of known diagnosis of HIV, HCV, and HBV; born in the United States; a resident of Baltimore City and adjacent counties of Maryland or the Maryland Eastern Shore; able to speak English well enough to be interviewed; noninstitutionalized; currently not taking antibiotics or immunosuppressive medications (steroids); and those who had undergone surgery provided a blood sample either before the surgery or 3 months after the surgery. Subjects who had undergone chemotherapy or radiation therapy were excluded from the study. Chemotherapy, radiation therapy, and active infections are known to affect the growth potential of the lymphocytes; thus, we excluded such subjects to maximize the validity of the results.

The study was approved by the Institutional Review Boards of the National Cancer Institute, University of Maryland, the Johns Hopkins University School of Medicine, Sinai Hospital, MedStar Research Institute, and the Research Ethics Committee of Bon Secours Baltimore Health System. After informed consent was obtained, cases and controls received a structured, in-person interview assessing prior medical and cancer history, tobacco use, alcohol use, current medications, occupational history, family medical history, menstrual history and estrogen use, recent nutritional supplements and caffeine intake, and socioeconomic characteristics. Blood was obtained by trained interviewers in heparinized tubes. Aliquots of the blood samples were transferred within 24 hours of collection to the Laboratory of Human Carcinogenesis at the National Cancer Institute for laboratory analyses. Laboratory personnel were masked to each participant's case-control status.

Blood cultures and preparation of chromosome spreads

Blood cultures were set up within 48 hours after the samples were obtained. One milliliter of fresh whole blood was added to 9 mL of RPMI 1640, supplemented with 15% fetal bovine serum (Biofluid, Inc., Rockville, MD), 1.5% of phytohemagglutinin (Invitrogen Co., Carlsbad, CA), 2 mmol/L L-glutamine, and 100 units/mL each of penicillin and streptomycin. After the cells were cultured for 90 hours at 37°C, the cells were exposed to 1.0 Gy γ-radiation at the rate of 1.26 Gy/min using a γ-irradiator (J.L. Shepherd, model Mark II) at room temperature and incubated at 37°C for an additional 3 hours. Colcemid (0.2 μg/mL) was added to the culture 2 hours after γ-radiation and incubated for an additional 1 hour at 37°C. The cells were treated in a hypotonic solution (0.06 mol/L KCl) for 25 minutes at room temperature and fixed in the fixative (3 parts of methanol with 1 part of acetic acid). The cells were dropped onto a clean microscopic slide, air-dried, and stained with 4% Gurr's Giemsa solution (Gallard Schlesinger, Carleplace, NY). We chose to measure the mitotic arrest at 3 hours after γ-radiation, because we are interested in testing the acute G_2-M checkpoint response to DNA damage and this time point has been used by others to study the G_2-M delay after γ-radiation of human cells (30). In addition, we consider the first 3 hours of the mitotic delay important, because it has been shown that >80% of the DNA damage was repaired within the first 3 hours after the exposure (31).

Mitotic index ascertainment

Giemsa-stained slides were examined using a Nikon Eclipse E400 microscope with 40 × objective. At least 1,000 cells were counted per subject and the percentage of metaphase cells was recorded. If less than five metaphase cells were found in 1,000 cells, then 5,000 cells were counted. If less than five metaphase cells were found in 5,000 cells in the untreated culture, the culture was regarded as failed (1.3% of the subjects) and the data were excluded from statistical analyses. The γ-radiation-induced G_2-M arrest was defined as the percentage of mitotic cells in the untreated culture minus the percentage of mitotic cells in the γ-radiation-treated culture from the same subject. The slides were coded and scored without the knowledge of case-control status.

Statistical analyses

The χ^2 goodness-of-fit test or Student's *t* test was used to examine the distributions of age, gender, race, and smoking status between cases and controls. γ-Radiation-induced G_2-M arrest was analyzed both as a continuous and categorical variable (i.e., quartiles of the response among the combined controls). Spearman's correlation was used to test the correlation between G_2-M arrest and age. In some analyses, the G_2-M arrest was categorized as "suboptimal" if the percentage of the G_2-M arrest was ≤1.40 (the 50th percentile value in controls). To assess for the presence of a trend in lung cancer risk according to the degree of G_2-M arrest, we then analyzed the data according to ordered categories, using the quartiles of the G_2-M arrest in the controls as cutoff points. Multivariate logistic regression was used to analyze the relationship between lung cancer risk and the G_2-M arrest phenotype, while controlling for other covariates. Baseline mitotic index, age, gender, race, and pack-years of smoking were covariates included in the multivariate analyses. In addition, interaction terms were included in the model and retained if their significance level was at least 0.01. Smoking status was stratified into three categories: never smokers, individuals who had never smoked >100 cigarettes in their life; former smokers, individuals who had smoked >100 cigarettes in their life, were not active smokers at the time of interview, and had quit >6 months before their interview; and current smokers, individuals who had smoked >100 cigarettes in their life, were active smokers at the time of interview, or had quit <6 months before their interview. All *P*s were two sided. All analyses were done using SAS software, version 9 (SAS Institute, Inc., Cary, NC).

Results

Study population

Table 1 summarizes selected demographic and exposure characteristics of the subjects. By design, our study has two control groups (hospital-based and population-based). Both control groups were cancer-free individuals recruited from the same catchment area as the cases. The case and control groups were well matched on some, but not all, sampling characteristics. Lung cancer patients and controls were similar in mean age and their gender distributions. African Americans were overrepresented in population controls ($P < 0.01$), which reflects our study design to oversampling African Americans (50%). The lung cancer cases were significantly more likely than the controls to be smokers ($P < 0.01$).

γ-Radiation-induced G_2-M arrest and lung cancer risk

The distribution of the percentage of γ-radiation-induced G_2-M arrest by case-control status is presented in Fig. 1. Overall, cases were significantly more likely to exhibit the low G_2-M arrest phenotype than were the control subjects (Fig. 1*A*). The case-control differences were more apparent among African Americans (Fig. 1*B*). The mean percentage of γ-radiation-induced G_2-M arrest was significantly lower in cases than in population controls (1.18 versus 1.44, $P < 0.01$) and hospital controls (1.18 versus 1.40, $P = 0.01$). Stratified analyses indicated that

significant case-control differences were present in African Americans and females (Table 2). Similar case-control differences were also present in Caucasians and males, but the differences did not reach statistical significance. When stratified by smoking status, the mean percentage of γ-radiation-induced G_2-M arrest was lower in cases than in controls in all three categories of smokers (never, former, and current; Table 2).

Interaction of γ-radiation-induced G_2-M arrest with host factors

Table 2 shows the mean percentage of γ-radiation-induced G_2-M arrest by various host factors and tobacco variables. G_2-M arrest was higher in females than males in both cases and controls, but the difference reached statistical significance only among population and combined controls groups ($P < 0.01$). Likewise, among the controls and to a lesser extent the cases, African Americans sustained greater levels of G_2-M arrest than Caucasians ($P < 0.01$). Age was not related to G_2-M arrest levels in cases, but among controls, the level was higher among older (mean G_2-M arrest = 1.54) than younger individuals (mean G_2-M arrest = 1.34, $P = 0.01$). Spearman correlation analysis indicated that γ-radiation-induced G_2-M arrest was significantly associated with age in controls ($r = 0.14$, $P = 0.01$), but not in lung cancer patients ($r = -0.10$, $P = 0.14$). When stratified by both race and gender, the controls were characterized by higher levels of G_2-M arrest among African American men when compared with Caucasian men and among African American women when compared with Caucasian women ($P < 0.01$). Also among the controls, older females sustained the highest levels of G_2-M arrest when compared with younger females and males. Neither smoking status, pack-years of smoking, nor years since quitting smoking were significantly associated with G_2-M arrest in either cases or controls (data not shown). Interaction terms were not statistically significant in the logistic regression models of lung cancer risk.

Risk estimates and dose-response trends

Univariate and multivariate analyses indicate that there were no significant differences between the two control groups in terms of G_2-M arrest (the mean percentage of G_2-M arrest was 1.40 for hospital controls and 1.44 for population controls, $P = 0.69$). Stratified analysis also indicated that the mean percentage of G_2-M arrest was similar between hospital controls and population controls within the race and gender subgroups ($P = 0.10$-0.50, Table 2). Therefore, in the subsequent analyses, these two control groups were combined to increase statistical power to detect case-control differences. We dichotomized data using the 50th percentile value in the controls (hospital controls and population controls pooled) as the cutoff. Decreased γ-radiation-induced G_2-M arrest was significantly associated with an increased risk of lung cancer after adjusting for age, gender, race, and pack-years of smoking [adjusted odd ratio (OR), 1.60; 95% confidence interval (95% CI), 1.10–2.33; Table 3, column aOR_1]. We then categorized the levels of G_2-M arrest according to quartiles of the G_2-M distribution in the controls. A significant trend of increasing lung cancer risk with decreased quartiles of G_2-M arrest was observed ($P_{trend} < 0.01$), with a lowest-versus-highest quartile adjusted OR of 2.15 (95% CI, 1.25–3.70). However, when the baseline mitotic index (percentage of mitotic cells in untreated cultures) was included in the logistic model, the association of γ-radiation-induced G_2-M arrest and lung cancer risk was not statistically significant in all subjects with an adjusted OR of 1.17 (95% CI, 0.73–1.88; Table 3, column aOR_2).

When the data were stratified by race, decreased γ-radiation-induced G_2-M arrest was associated with an increased risk of lung cancer in African Americans (adjusted OR_2, 2.25; 95% CI, 0.97–5.20), adjusted for baseline mitotic index (continuous), age, gender, and pack-years of smoking. A significant trend of an increased lung cancer risk with decreased quartiles of G_2-M arrest was observed in African Americans ($P_{trend} = 0.02$), with a lowest-versus-highest quartile aOR_2 of 3.74 (95% CI, 0.98–14.3). Decreased G_2-M arrest was not associated with an increased risk of lung cancer in Caucasians.

When the data were stratified by both gender and decreased γ-radiation-induced G_2-M arrest was significantly associated with an increased risk of lung cancer in African American females (adjusted OR_2, 5.19; 95% CI, 1.33–20.19), adjusted for baseline mitotic index, age, and pack-years of smoking. A significant trend of an increased lung cancer risk with decreased quartiles of G_2-M arrest was also observed in African American females ($P_{trend} < 0.01$), with a lowest-versus-highest quartile adjusted OR of 11.75 (95% CI, 1.47–94.04). However, an association of G_2-M arrest and lung cancer risk was not significant in both Caucasian and African American males nor in Caucasian females (Table 3).

Discussion

In this study, we showed that reduced efficiency in the function of DNA damage–induced G_2-M cell cycle checkpoint was associated with an increased risk of lung cancer among African Americans. The failure to maintain genomic integrity is central to the problem of carcinogenesis. Increased genetic instability, either spontaneous or mutagen induced, has been considered a predisposing factor for neoplastic transformation. To ensure the high-fidelity transmission of genetic information, cells have evolved mechanisms to monitor genome integrity. Cells respond to DNA damage by activating a complex DNA-damage-response pathway that includes cell cycle arrest, DNA repair, and apoptosis. Cell cycle checkpoints are mechanisms that regulate progression though the cell cycle, ensuring that each step takes place only once and in the right sequence. It has long been known that DNA-damaging agents induce a cell cycle arrest, buying time for repair, and thus protecting the organism from the deleterious consequences of mutation (24,32).

The DNA damage checkpoints act at three different stages of the cell cycle, inducing G1 arrest, blockage of DNA replication, or G_2 delay, depending on the type of damage and cell cycle stage when the damage was detected. If the G_2-M arrest fails, the broken chromosome may be subjected to mitosis and the damaged chromosomes may be partitioned into separate nuclei. This situation can lead to genomic instability, which in turn, may enhance the rate of cancer development. This is supported by the observation that individuals with ataxia telangiectasia syndrome and Nijmegen breakage syndrome, which all show defects in DNA repair/cell cycle control and increased genomic instability, are prone to cancer (33,34). Previous studies also suggest that individuals who have inherited mutations in genes involved in the G_2 checkpoint and DNA damage repair are predisposed to the development of various types of cancer, and that their cells have a strong tendency to accumulate additional mutations (35). In addition, the signaling pathway leading to G_2 arrest after DNA damage is frequently altered or mutated in human cancers (9,10,36–38). Therefore, individuals with a deficiency in the G_2-M checkpoint may be predisposed to lung cancer. Our data are consistent with this hypothesis. We found that the mean percentage of γ-radiation-induced G_2-M arrest was significantly higher in our controls than in lung cancer cases. In addition, a trend of an increased lung cancer risk was associated with decreased efficiency of G_2-M arrest among African Americans.

Our data also indicated that the association between γ-radiation-induced G_2-M arrest and lung cancer risk was restricted to African Americans and the differences associated with race were almost entirely due to the African American control group having a mean percentage of γ-radiation-induced G_2-M arrest that was 34% higher than the Caucasian control group. There could be several explanations for this observation. One is because of potential biological differences. There is evidence to indicate that random distribution of allele frequencies throughout the human genome follows diverse ethnic and/or racial trends (39,40). The frequency of sequence variation can differ by race and ethnicity, and this variation may be associated with a difference in risk for disease between these groups (41). For example, documented differences in allele frequencies between African Americans and Caucasians for genes involved in DNA repair (42) and hormone metabolism (43) have been proposed to

contribute to differences in lung cancer (44), breast cancer (42,45), and prostate cancer (46) risk. There are studies indicating that the prevalence of some cancer susceptibility polymorphisms [i.e., poly(ADP-ribose) polymerase, *p53*, and *CYP1A1*] are significantly dependent on ethnicity (44,47,48). Differences in allele frequencies between African Americans and Caucasians were reported in genes that have been implicated in immune responses to tumors (49). It is possible that a less-efficient G_2-M checkpoint function is a significant host factor for lung cancer susceptibility in African Americans.

Phenotypic biomarkers that measure at risk biological responses could be potentially more useful as intermediate indicators of cancer risk than single nucleotide polymorphisms (SNP). It is not well understood how the common, low-penetrance genes involving cell cycle checkpoints contribute to the lung cancer susceptibility, partly because data on genetic polymorphisms involving cell cycle control genes are sparse. Reports on the association of *p53* and *Chk2* gene polymorphisms and lung cancer risk are inconsistent (50–53). SNPs are often considered indicators of genetic risks across individuals. However, in most instances, a SNP merely reflects a variation in the DNA sequence of a given gene in an individual, or has a moderate effect on disease risk. This may explain the abundance of conflicting SNP studies reported in the literature. If many different genes play a role in a given pathway to produce an at-risk biological response, functional measures of this biological response would be more effective in predicting the disease risk than the SNPs themselves. However, many potential pitfalls of phenotypic markers need to be considered carefully. The measurement of biological function at one time can be influenced by many endogenous and exogenous factors, such as medication, hormone levels, disease status, and treatment. The proliferating potential of the lymphocytes can be a powerful factor affecting the measurement of cell cycle in cultures. We have carefully considered many of the potential factors that could confound the measurement of cell cycle checkpoint by study design and analysis. Our study has very strict exclusion criteria to exclude subjects who have had chemotherapy, radiation therapy, and active infection and who were current users of immunosuppressive drugs (see eligibility criteria in Materials and Methods for detail). However, we still observed significant differences in baseline mitotic index, which is a good indicator of cell growth, between cases (mean baseline mitotic index = 2.02) and controls (mean baseline mitotic index = 2.30, $P < 0.01$). Therefore, we also included baseline mitotic index in the logistic model to account for any residual confounding by factors that may influence cell growth. We also did the analyses using the alternative definition of G_2-M arrest [G2-M arrest = (the baseline mitotic index − the mitotic index in γ-radiation–treated culture) /baseline mitotic cell index] to account for the effect of baseline mitotic index and consistently found that deficiency in G_2-M arrest was associated with an increased risk of lung cancer in African Americans (data not shown).

Our study used a phenotypic marker to measure the function of the G_2-M checkpoint and showed that deficiencies in the G_2-M checkpoint contribute to an elevated lung cancer risk in African Americans. This study provides the first molecular checkpoint is associated with lung cancer risk among African Americans. If replicated, these results may provide clues to the exceedingly high lung cancer incidence experienced by African Americans.

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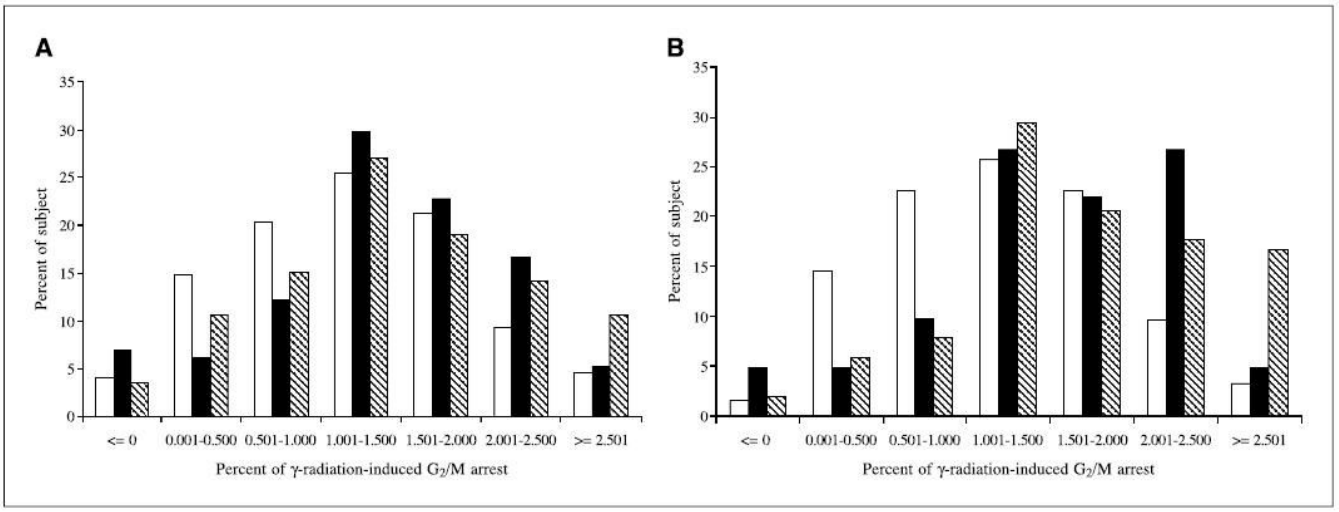

Figure 1.

Distribution of γ-radiation-induced G_2-M arrest by case-control status in all subjects (*A*) and in African Americans (*B*). The cases were more likely to exhibit the low G_2-M arrest phenotype (<1.5%) than were the control subjects, and the case-control differences were more apparent among African Americans. □, cases; ▪, hospital controls; ▧, population controls.

Table 1

Distribution of selected characteristics of study subjects

	Cases (*n* = 216)	Population controls (*n* = 226)	*P**	Hospital controls (*n* = 114)	*P*†
Age (y), mean (SD)	65.9 (10.4)	65.1 (9.5)	0.40	63.7 (12.1)	0.09
Male	65.8 (9.9)	66.4 (8.3)	0.66	69.4 (9.2)	0.03
Female	65.9 (10.8)	63.7 (10.5)	0.13	59.3 (12.4)	<0.01
Gender, *n*(%)					
Male	105 (48.6)	116 (51.3)	0.57	50 (43.5)	0.41
Female	111 (51.4)	110 (48.7)		64 (56.1)	
Race, *n*(%)					
African American	62 (28.7)	102 (45.1)	<0.01	41 (36.0)	0.18
Caucasian	154 (71.3)	124 (54.9)		73 (64.0)	
Smoking status, *n*(%)					
Never	13 (6.0)	88 (38.9)	<0.01	22 (19.3)	<0.01
Former smoker	84 (38.9)	107 (47.4)		56 (49.1)	
Current smoker	119 (55.1)	31 (13.7)		36 (31.6)	
Pack-years, *n*(%)					
≤20	47 (21.8)	159 (70.4)	<0.01	41 (35.9)	0.02
21–40	69 (31.9)	36 (15.9)		25 (21.9)	
41–60	58 (26.9)	25 (11.1)		23 (20.2)	
>60	42 (19.4)	6 (2.7)		25 (21.9)	
Menopausal status, *n*(%)					
Premenopausal	6 (5.4)	12 (10.9)	0.13	17 (26.6)	<0.01
Postmenopausal	105 (94.6)	98 (89.1)		47 (73.4)	

* For comparison between cases and population controls.

† For comparison between cases and hospital controls.

Table 2

Mean percent γ-radiation-induced G_2-M arrest by host characteristics

Variable	Cases		Population control			Hospital control			Combined controls*		
	n	Mean (SD)	*n*	Mean (SD)	*P*	*n*	Mean (SD)	*P*	*n*	Mean (SD)	*P*
Total	216	1.18 (0.78)	226	1.44 (0.83)	<0.01	114	1.40 (0.87)	0.01	340	1.43 (0.85)	<0.01
Gender											
Male	105	1.14 (0.76)	116	1.26 (0.82)	0.41	50	1.38 (0.90)	0.08	166	1.30 (0.85)	0.11
Female	111	1.22 (0.80)	110	1.63 (0.81)	<0.01	64	1.41 (0.86)	0.07	174	1.55 (0.83)	<0.01
P		0.57		<0.01			0.54			<0.01	
Race											
AA†	62	1.23 (0.80)	102	1.71 (0.84)	<0.01	41	1.57 (0.76)	0.02	143	1.67 (0.82)	<0.01
Caucasian	154	1.16 (0.77)	124	1.22 (0.77)	0.59	73	1.31 (0.92)	0.16	197	1.25 (0.83)	0.27
P		0.67		<0.01			0.07			<0.01	
Gender and race											
AA male	29	1.13 (0.75)	49	1.55 (0.90)	0.04	13	1.32 (0.56)	0.43	62	1.50 (0.80)	0.05
AA female	33	1.31 (0.85)	53	1.86 (0.75)	<0.01	28	1.69 (0.82)	0.08	81	1.80 (0.78)	<0.01
CA male‡	76	1.14 (0.76)	67	1.05 (0.68)	0.50	37	1.41 (0.99)	0.12	104	1.18 (0.82)	0.73
CA female	78	1.18 (0.79)	57	1.42 (0.80)	0.09	36	1.20 (0.83)	0.88	93	1.33 (0.82)	0.21
P		0.75		<0.01			0.17			<0.01	
Smoking status											
Never	13	1.06 (0.64)	88	1.55 (0.77)	0.04	22	1.23 (0.91)	0.43	110	1.49 (0.80)	0.04
Former smoker	84	1.19 (0.77)	107	1.39 (0.87)	0.17	56	1.36 (0.79)	0.19	163	1.38 (0.84)	0.08
Current	119	1.18 (0.80)	31	1.29 (0.87)	0.60	36	1.57 (0.95)	0.01	67	1.44 (0.92)	0.05
P		0.81		0.18			0.42			0.59	

*Combined controls = population controls plus hospital controls.

†African American.

‡Caucasian.

Table 3

Risk estimates for γ-radiation-induced G_2-M arrest

G_2-M arrest	Cases, *n* (%)	Combined controls, *n* (%)	AOR_1* (95% CI)	aOR_2† (95% CI)
Total				
Above the median	82 (38.0)	170 (50.0)	1.0 (reference)	1.0 (reference)
Below the median	134 (62.0)	170 (50.0)	1.60 (1.10–2.33)	1.17 (0.73–1.88)
By quartiles				
Fourth	33 (15.3)	85 (25.0)	1.0 (reference)	1.0 (reference)
Third	49 (22.7)	85 (25.0)	1.26 (0.71–2.23)	1.03 (0.55–1.92)
Second	52 (24.0)	85 (25.0)	1.48 (0.84–2.60)	1.08 (0.55–2.13)
First	82 (38.0)	85 (25.0)	2.15 (1.25–3.70)	1.41 (0.66–3.02)
P_{trend}			<0.01	0.31
African Americans				
Above the median	22 (35.5)	89 (62.2)	1.0 (reference)	1.0 (reference)
Below the median	40 (64.5)	54 (37.8)	2.43 (1.23–4.79)	2.25 (0.97–5.20)
By quartiles				
Fourth	11 (17.7)	50 (35.0)	1.0 (reference)	1.0 (reference)
Third	11 (17.7)	39 (27.3)	0.93 (0.33–2.64)	0.95 (0.30–3.01)
Second	18 (29.0)	31 (21.7)	1.55 (0.59–4.07)	1.60 (0.49–5.22)
First	22 (35.5)	23 (16.0)	3.58 (1.39–9.25)	3.74 (0.98–14.3)
P_{trend}			<0.01	0.02
African American males				
Above the median	11 (37.9)	34 (54.8)	1.0 (reference)	1.0 (reference)
Below the median	18 (62.1)	28 (45.2)	1.60 (0.59–4.30)	1.32 (0.43–4.08)
By quartiles				
Fourth	4 (13.8)	14 (22.6)	1.0 (reference)	1.0 (reference)
Third	7 (24.1)	20 (32.3)	1.13 (0.24–5.31)	0.98 (0.18–5.32)
Second	9 (31.0)	14 (22.6)	1.28 (0.27–5.99)	1.08 (0.19–6.14)
First	9 (31.0)	14 (22.6)	2.31 (0.51–10.57)	1.76 (0.24–12.76)
P_{trend}			0.25	0.51
African American females				
Above the median	11 (33.3)	55 (67.9)	1.0 (reference)	1.0 (reference)
Below the median	22 (66.7)	26 (32.1)	3.69 (1.41–9.71)	5.19 (1.33–20.19)
By quartiles				
Fourth	7 (21.2)	36 (44.4)	1.0 (reference)	1.0 (reference)
Third	4 (12.1)	19 (23.5)	0.62 (0.13–2.99)	0.91 (0.16–5.16)
Second	9 (27.3)	17 (21.0)	1.92 (0.54–6.85)	3.45 (0.58–20.40)
First	13 (39.4)	9 (11.1)	5.45 (1.50–19.87)	11.75 (1.47–94.04)
P_{trend}			<0.01	<0.01
Caucasians				
Above the median	60 (39.0)	81 (41.1)	1.0 (reference)	1.0 (reference)
Below the median	94 (61.0)	116 (58.9)	1.23 (0.78–1.95)	0.78 (0.44–1.40)
By quartiles				
Fourth	22 (14.3)	35 (17.7)	1.0 (reference)	1.0 (reference)
Third	38 (24.7)	46 (23.4)	1.27 (0.62–2.60)	0.91 (0.42–1.99)
Second	34 (22.0)	54 (27.4)	1.21 (0.59–2.48)	0.70 (0.29–1.66)
First	59 (39.0)	62 (31.5)	1.60 (0.82–3.14)	0.77 (0.30–1.99)
P_{trend}			0.19	0.53
Caucasian males				
Above the median	27 (35.5)	36 (34.6)	1.0 (reference)	1.0 (reference)
Below the median	49 (64.5)	68 (65.4)	1.13 (0.58–2.18)	0.80 (0.35–1.81)
By quartiles				
Fourth	10 (13.2)	15 (14.4)	1.0 (reference)	1.0 (reference)
Third	17 (22.4)	21 (20.2)	1.33 (0.45–3.93)	1.06 (0.33–3.40)
Second	18 (23.7)	34 (32.7)	1.09 (0.38–3.09)	0.78 (0.23–2.64)
First	31 (40.8)	34 (32.7)	1.59 (0.58–4.34)	0.96 (0.24–3.83)
P_{trend}			0.41	0.86
Caucasian females				
Above the median	33 (42.3)	45 (48.4)	1.0 (reference)	1.0 (reference)
Below the median	45 (57.7)	48 (51.6)	1.30 (0.68–2.49)	0.78 (0.33–1.82)
By quartiles				
Fourth	12 (15.4)	20 (21.5)	1.0 (reference)	1.0 (reference)
Third	21 (26.9)	25 (26.9)	1.11 (0.42–2.96)	0.72 (0.24–2.10)
Second	16 (20.5)	20 (21.5)	1.37 (0.48–3.89)	0.65 (0.18–2.33)
First	29 (37.2)	28 (30.1)	1.40 (0.55–3.57)	0.54 (0.14–2.07)
P_{trend}			0.43	0.40
Males				
Above the median	38 (36.2)	70 (42.2)	1.0 (reference)	1.0 (reference)
Below the median	67 (63.8)	96 (57.8)	1.29 (0.75–2.22)	0.97 (0.51–1.87)
By quartiles				
Fourth	14 (13.3)	28 (16.9)	1.0 (reference)	1.0 (reference)
Third	24 (22.9)	42 (25.3)	1.19 (0.50–2.83)	0.96 (0.37–2.46)
Second	27 (25.7)	48 (28.9)	1.21 (0.52–2.83)	0.89 (0.33–2.37)

G_2-M arrest	Cases, *n* (%)	Combined controls, *n* (%)	AOR_1* (95% CI)	aOR_2† (95% CI)
First	40 (38.1)	48 (28.9)	1.66 (0.73–3.78)	1.06 (0.34–3.24)
P_{trend}			0.21	0.91
Females				
Above the median	44 (39.6)	100 (57.5)	1.0 (reference)	1.0 (reference)
Below the median	67 (60.4)	74 (42.5)	1.93 (1.13–3.27)	1.49 (0.75–3.00)
By quartiles				
Fourth	19 (17.1)	57 (32.8)	1.0 (reference)	1.0 (reference)
Third	25 (22.5)	43 (24.8)	1.13 (0.52–2.48)	0.92 (0.39–2.19)
Second	25 (22.5)	37 (21.2)	1.71 (0.78–3.77)	1.26 (0.46–3.40)
First	42 (37.8)	37 (21.2)	2.35 (1.12–4.92)	1.60 (0.55–4.63)
P_{trend}			0.01	0.26

*Adjusted for age, pack-years, race, and/or gender.

†Adjusted for baseline mitotic index (continuous), age, pack-years, race, and/or gender.