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DNA damage phenotype and prostate cancer risk

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Abstract

The capacity of an individual to process DNA damage is considered a crucial factor in carcinogenesis. The comet assay is a phenotypic measure of the combined effects of sensitivity to a mutagen exposure and repair capacity. In this paper, we evaluate the association of the DNA repair kinetics, as measured by the comet assay, with prostate cancer risk. In a pilot study of 55 men with prostate cancer, 53 men without the disease, and 71 men free of cancer at biopsy, we investigated the association of DNA damage with prostate cancer risk at early (0-15 min) and later (15-45 min) stages following gamma-radiation exposure. Although residual damage within 45 min was the same for all groups (65% of DNA in comet tail disappeared), prostate cancer cases had a slower first phase (38% vs 41%) and faster second phase (27% vs 22%) of the repair response compared to controls. When subjects were categorized into quartiles, according to efficiency of repairing DNA damage, high repair-efficiency within the first 15 min after exposure was not associated with prostate cancer risk while higher at the 15-45 min period was associated with increased risk (OR for highest-to-lowest quartiles = 3.24, 95% CI=0.98-10.66, *p*-trend =0.04). Despite limited sample size, our data suggest that DNA repair kinetics marginally differ between prostate cancer cases and controls. This small difference could be associated with differential responses to DNA damage among susceptible individuals.

Keywords

prostate cancer; benign prostatic hyperplasia; comet assay; DNA repair; case-control study

1. Introduction

Prostate cancer is the second leading cause of cancer death among men in the United States [1]. Despite its high morbidity, the only established risk factors for the disease are race, family history of prostate cancer, advancing age, and, more recently (from genome wide association studies), genetic variants in the chromosome 8q24 region. Germline mutations or polymorphisms in genes that regulate cell cycle control and DNA repair, *e.g.*, *BRCA1/1*,

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CHEK2, *XRCC1*, and *OGG1*, have been associated with prostate cancer risk [2-5], suggesting that deficiencies in these pathways may play a significant role in individual susceptibility to prostate cancer. These results, combined with strong biological plausibility, suggest that defects in DNA repair mechanisms may influence susceptibility [2,6-10].

Phenotypic cell-based assays of repair capacity provide a quantitative view of the complex pathways involved in repair [11]. The comet assay detects DNA damage at the single-cell level, in terms of migration of the DNA out of the nucleus under alkaline electrophoretic conditions [12]. As such, it serves as a phenotypic assay of the combined effects of sensitivity to the mutagen exposure and the individual's DNA damage response. Simple modification of the comet assay allows the measurement of DNA repair capacity and, in combination with the analysis of polymorphisms in relevant genes, the assay can provide important information on interactions between genetic variation and environmental exposures in maintaining genomic stability [13]. A pilot study by our group showed that quantitation of repair in whole blood, following gamma radiation challenge, is feasible, and we detected slower repair among head and neck cancer patients [14].

The comet assay has been used as a biological marker of cancer susceptibility in human peripheral lymphocytes in several case-control studies and was shown to be an independent risk factor for several cancers, including bladder, breast, cervical, lung and esophageal [15-19]. For prostate cancer, one study showed that above-median H₂O₂ – induced DNA damage is associated with a non-significant 60% increase in prostate cancer risk [20]. The study did not, however, estimate the DNA repair kinetics (only the composite damage/repair measure). In this report, we evaluated the association of DNA repair kinetics, measured as residual damage in the comet tail, with prostate cancer risk in whole blood human cultures; gamma radiation was the mutagen. We hypothesized that patients with prostate cancer would have slower DNA repair mechanisms, measured as more residual DNA damage, compared to cancer-free individuals.

2. Materials and Methods

2.1 Sample collection and processing

The 55 prostate cancer cases were recruited at Georgetown University Medical Center, Departments of Urology and Radiation Oncology and the Veterans Administration (VA) Medical Center, Department of Urology, in the Washington DC area between 2004 and 2007. All prostate cancer cases were confirmed by biopsy and enrolled prior to the initiation of treatment. Seventy percent of cases had Gleason scores ≤ 6 , 25% had Gleason score > 7 and information was missing for three cases. Healthy controls (N=53) were prostate disease-free men not related to prostate cases. These participants accompanied other patients (n=7), visited for routine checkups, non-prostate issues (e.g. testicular pain, erectile dysfunction, bladder stone analysis) (n=8); or participated in the National Lung Screening Trial (a multicenter study of lung cancer risk sponsored by the NCI) (n=38). Biopsy controls (N=71) were patients who were confirmed prostate cancer-free at biopsy and were associated with urological conditions, typically benign prostatic hyperplasia (BPH). The percentages of subjects recruited at the VA site were 2% (cases) and 3% (biopsy controls). Participation rates among the eligible subjects were 57% (cases), 68% (healthy controls), and 43% (biopsy controls). Eligible subjects had to have no prior history of cancer and be able to speak English well enough to be interviewed. The study was approved by the MedStar Research Institute-Georgetown University Oncology Institutional Review Board.

After informed consent was obtained, cases and controls received a structured, in-person interview assessing prior medical history, tobacco and alcohol use, current medications, family medical history, and socioeconomic characteristics. Blood samples were drawn in

green-topped Vacutainer tubes (BD BioSciences, Franklin Lakes, NJ) containing sodium heparin (10-25 IU/mL). Whole blood used for the comet assay was diluted 1:10 in RPMI 1640 prior to embedding in agarose.

2.2 Alkaline comet assay

The alkaline comet assay protocol was conducted in accordance with the guidelines summarized by Tice et al. [21] and modified by our group as described previously [14]. Briefly, frosted slides (Thermo-Fisher, Waltham, MA) were coated with 1% agarose (Sigma-Aldrich, St. Louis, MO) and dried in an oven at 60°C for 20 min. The second layer consisted of 0.75% normal-melting agarose (Invitrogen, Gaithersburg, MD). Cells were mixed with 0.75% low-melting agarose (BioWhittaker-Cambrex, East Rutherford, NJ) and kept at 37°C under a yellow-frosted incandescent light (Philips Electronics North America, New York, NY) to prevent DNA damage. Cells were embedded in the topmost layer and the second and the third layers were flattened using a 22×22mm coverslip (Thermo-Fisher, Waltham, MA) on ice to facilitate the gelling process. Duplicate slides were made for each dose/repair point. After the embedding procedure, the cells were exposed to 9 Gy gamma radiation derived from a ¹³⁷Cs source in a Research Irradiator, to generate DNA damage, followed either by lysis in buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 200 mM NaOH, 1% (v/v) Triton X-100, 10% (v/v) DMSO, adjusted to pH 10) or repair in RPMI 1640 medium at 37°C for a fixed length of time (15 or 45 min). Slides were then transferred to lysis buffer at 4°C in the dark for 3-24 h. The slides were transferred to ice cold electrophoresis buffer (pH=13; 300 mM NaOH, 1mM EDTA) for 40 min. Electrophoresis was carried out at 13 V/cm for 45 min at 4°C in the dark. The volume of the buffer was adjusted so that the current at the start was 300 mA. Slides were neutralized using three 5-min washes with autoclaved 0.4M Tris buffer (pH 7.4). DNA was fixed with methanol for 10 min. Slides were then washed with autoclaved distilled water twice for 5 min and allowed to dry overnight. The dried slides were kept in a storage box (Thermo-Fisher, Waltham, MA) until scoring. All slides were treated similarly, to avoid assay variability that would potentially bias the results.

2.3 Comet Scoring and data collection

All experimental procedures were performed without knowledge of the case/control status. Slides were rehydrated for 45-60 min in autoclaved distilled water and stained for 10 min using ethidium bromide solution, 1µg/ml (Invitrogen, Gaithersburg, MD). Excess stain was washed away using three 5 min washes with autoclaved distilled water. Slides were scored wet with a cover glass over the gel. Comet images were obtained using an Olympus BC-51 microscope (Opelco-Olympus, Center Valley, PA) equipped with a 100W mercury burner and a wide green fluorescent mirror (U-MWG2) and a cooled 5 megapixel digital CCD camera (QImaging Micropublisher 5.0 RTV, QImaging, Surrey, BC, Canada). Two neutral density filters (U-25ND25 and U-25ND50) were inserted into the light path to reduce photobleaching of the fluorophore. Images were scored using a semi-automated Comet Analysis System (Loats Associates, Westminster, MD). At least 50 images were recorded at 400× total magnification for each slide, with two slides per dose-repair point. In accordance with the idea that 'hedgehogs' are dead cells that do not offer information regarding DNA repair [22], we tracked them as a categorical variable but did not include them in the calculation of the summary statistics. We observed 2.1 'hedgehogs' per 100 cells, on average. The Loats software calculated a number of parameters but we focused primarily on percent DNA in tail, the most reliable parameter for inter-laboratory comparisons [23].

One hundred comet images were recorded for each dose-repair point (two slides, 50 images per slide). These images were analyzed by a semi-automated scoring system (Loats, Associates, Westminster, MD). The percent DNA in tail for each image was used as the

variable of interest. Percent repaired was calculated as follows: $[(\text{Initial damage} - \text{Damage at time T}) / \text{initial damage}] \times 100$. Percent repaired is considered a complement of the measured percent of residual damage in the comet tail.

2.4 Statistical Analysis

The Chi-square goodness-of-fit test or Student's t-test was used to examine the distributions of age, smoking status, presence of BPH, and other parameters of interest, between cases and controls. Subjects were categorized in two groups based on their smoking status: never/former smokers and smokers. Never/former smokers consist of individuals who had never smoked more than 100 cigarettes in their life or who had smoked more than 100 cigarettes in their life and have not smoked for a year or longer. Smokers have smoked more than 100 cigarettes in their lifetime and have smoked in the past year. Positive family history of prostate cancer was defined as having a father, brother, uncle, or cousin diagnosed with prostate cancer. Information on the presence of BPH was self-reported, based on whether the subject responded positively to any of the following questions: ever diagnosed with an enlarged prostate?; wake up two times or more every night to urinate? Presence of BPH diagnosis, as described above, was in 100% concordance with the presence of BPH based on pathology reports, for subjects with available medical history. However, as there were no medical records available for 35% of the study population (primarily men of the healthy control group), we chose to define BPH based on the criteria described above, in these individuals.

Non-parametric statistics were used to compare case and control groups with respect to DNA repair and multivariate logistic models were used to assess the risk estimates in the different DNA repair profile categories, adjusting for age, race, presence of BPH, smoking status and body mass index (BMI). DNA damage (%DNA in tail) following irradiation was categorized by quartiles based on the comet scores, with the lowest quartile representing hyposensitive, the middle quartiles representing sensitive, and the highest quartile representing hypersensitivity; for trend analysis, an ordinal variable based on these categories was used, with 1 representing hyposensitive and 4 representing hypersensitive. The lowest quartile was used as a reference. The degree of DNA repair following gamma radiation exposure, were modeled similarly, with the lowest quartile representing hypo-efficient (lowest quartile), middle two quartiles representing efficient, and highest quartile representing hyper-efficient, based on their comet scores; hypo-efficient men (the lowest quartile) were used as the reference and an ordinal variable based on these categories was used for trend tests. All *P* values were two-sided and considered significant if $p < 0.05$. All analyses were performed using SAS software, version 9 (SAS Institute Inc., Cary, NC).

3. Results

Our study included three groups; prostate cancer cases, healthy controls, and biopsy controls [subjects who underwent biopsy primarily due to the elevated prostate specific antigen (PSA) and were confirmed to be cancer-free]. Demographic characteristics of the study participants are presented in Table 1. Healthy controls (mean age 61) tended to be younger than prostate cancer cases and biopsy controls (mean age 65 for both groups; $p=0.01$), were predominantly Caucasian (96%), as compared with 73% of prostate cases ($p=0.01$) and 54% of biopsy controls ($p<0.01$), and would more often report to be current or former smokers (82%) compared to both cases and biopsy controls (52%; $p<0.01$ and 53%, $p<0.01$ respectively, when compared to healthy controls). Among biopsy controls, 77% presented with symptoms of benign prostatic hyperplasia, compared to 60% of healthy controls and 70% of prostate cases. High PSA (≥ 4 ng/ml) was reported among 73% of cases and 70% of biopsy controls, compared to 17% among healthy controls. Cases did not differ from the

control groups with regards to family history of prostate cancer. Biopsy controls reported lower household incomes compared to both cases and healthy controls ($p<0.01$).

We first evaluated whether DNA damage and DNA repair kinetics differed between prostate cancer cases and healthy and biopsy controls (Table 2). Before any treatment, basal damage levels (% DNA in tail) were comparable among cases, biopsy controls and healthy controls (1% for all groups). Cases and both control groups showed comparable initial damage following exposure to 9 Gy of gamma radiation, and at 15 and 45 minutes following exposure, when measurements were corrected for basal damage. Comparable to a previous study [24], the repair kinetic appeared biphasic, with faster repair within the first 15 min and slower repair from 15-45 min. Prostate cases and the two control groups repaired approximately 65% of the damage within 45 min; in other words, there was 35% residual damage at that time point for all groups. We observed that prostate cases would repair less of the DNA during the first phase of repair (0-15 min) but more of the DNA during the second phase of repair (15-45 minutes) compared to the control groups. In more detail, 15 min following irradiation, prostate cancer cases had 38% of the damage repaired, compared to 42 % for healthy controls and 40 % for biopsy controls. At the 15-45 min period following gamma radiation exposure, cases would repair 27 % of the damage compared to 22 % for healthy controls and 23 % for biopsy controls. These differences between groups were not statistically significant.

To evaluate the potential determinants of DNA repair kinetics, we assessed the association between different covariates and DNA repair kinetics in the healthy control group (Table 3), choosing age 60 as a cutoff to define “younger” versus “older” men. We did not find any differences in DNA repair with respect to age. With respect to smoking status, the percent DNA in tail immediately following exposure to gamma radiation was higher among current smokers than among never/former smokers (38% vs 46%, respectively; $p=0.04$). Moreover, the percent repair of DNA in tail between 0-45 minutes was higher among current smokers than never/former smokers (70 % vs 62 %, respectively; $p=0.05$) and the difference seemed to be driven by the difference in the fast phase of the repair kinetics, within 15 min after exposure. Being overweight ($BMI \geq 25$) was correlated with a slight increase in percent DNA in tail at the 15 min time point following exposure ($p=0.04$). Neither high PSA (≥ 4 ng/ml) nor presence of BPH was associated with the DNA repair profile. As 96% of the healthy population participants were Caucasian, we were unable to examine the effect of race on the repair kinetics. Among prostate cancer patients, we also evaluated whether disease aggressiveness, as determined by Gleason score and tumor stage, was related to DNA damage. Our results showed that having a high Gleason score (>7) did not affect the patients’ DNA repair profile. Similarly, neither basal nor gamma radiation induced DNA damage levels differed by tumor stage (results not shown). Staging information was available for only half (25 out of 55) of the prostate cancer cases. None of the covariates examined had an effect on baseline DNA damage at time 0 (results not shown).

We used multivariate logistic regression models to examine the association of prostate cancer and DNA damage and DNA repair kinetics; all models were adjusted for age, race, presence of BPH, smoking status, and BMI (Table 4). For this analysis, healthy controls and biopsy controls were combined, since the repair kinetics of the two groups were comparable. We were not able to conclude whether subjects with hypersensitivity to gamma radiation at 0, 15 and 45 min following exposure would be at increased risk of developing prostate cancer, since none of the risk estimates was statistically significant. However, suggestive increases in risk were seen for hypersensitive men as compared to hyposensitive men for time 0 after exposure ($OR=1.71$, 95% $CI=0.53-5.51$), for time 15 minutes after exposure ($OR=1.56$, 95% $CI=0.43-5.72$), and for time 45 min following exposure ($OR=1.25$, 95% $CI=0.34-4.52$). When subjects were categorized according to degree of efficiency of

repairing DNA damage, being hyper-efficient in repairing DNA damage within the first 15 min was suggestive of being protective against prostate cancer (adjusted OR = 0.38, 95% CI =0.10-1.37, p-trend for highest-to-lowest quartile=0.11). Moreover, being a case was associated with slightly higher efficiency in repairing DNA damage at the 15-45 min period following irradiation (OR for highest-to-lowest quartile = 3.24, 95% CI=0.98-10.66, p-trend =0.04). Similar results were obtained if analysis was performed for healthy controls and prostate cancer cases or biopsy controls and prostate cancer cases separately (data not shown).

4. Discussion

In this report, we examined the kinetics of DNA repair, estimated by the comet assay, in prostate cancer. We show that DNA repair kinetics following irradiation are only marginally different between prostate cancer cases and controls. More specifically, we observed that prostate cancer cases repair DNA damage slightly less efficiently in the first 15 min following exposure to gamma radiation, but slightly more efficiently at the later stage of recovery. We do not, however, observe an overall difference in repair or residual damage; cases and controls repair approximately 65% of the DNA damage in 45 min (35% residual damage at that time point). Slower repair in the first 15 min was expected but we did not expect to observe the faster repair in cases in the later phase.

A previous report on the application of the comet assay to prostate cancer risk showed that compromised DNA repair machinery is associated with prostate cancer risk [20]. The report did not, however, estimate the DNA repair kinetics (only the composite damage/repair measure), and used H₂O₂ as the mutagen. It is possible that radiation and H₂O₂ insults are repaired by distinct pathways. Therefore, it may be necessary to use a panel of mutagens and additional repair assays to assess in more detail the DNA repair phenotypes. Moreover, we studied repair in fresh whole blood cultures, as opposed to the primary cultured lymphocytes that were used in the previous report. In a comparative analysis performed in our laboratory [14], we estimated that the initial damage following gamma irradiation was higher in stimulated lymphocytes compared to whole blood cultures, possibly due to the higher fraction of the cells at G₀. However subsequent repair was comparable in the two cell preparations.

Given the common risk factors between BPH and prostate cancer (advancing age, hormone dependence, may be related to inflammation), and that they often coexist [25], we aimed to evaluate whether men with BPH and elevated PSA were less susceptible to prostate cancer because of their more efficient DNA repair mechanisms by inclusion of a biopsy control group who were cancer-free, had elevated PSA, and most of whom had BPH. The data did not confirm our hypothesis and the two control groups were combined in the analysis of risk estimations.

We chose to use gamma radiation as the mutagen and measured DNA damage following a single exposure to 9 Gy and tested the DNA repair kinetics at two time points (15 and 45 min), to approximate the fast and slow portion of the repair [15,24]; gamma radiation has an advantage over chemical agents in that it is less subject to individual variations in uptake, metabolism, and detoxication [26]. Using normal human lymphocytes, the types of DNA strand break changes with post-irradiation time were demonstrated [27]. In the early stages (0-30 min) gamma radiation induced the formation of single-strand breaks. After 1 h, there was an increase in double-strand breaks. Single-strand breaks are removed by base excision repair mechanisms [28,29], while double-strand breaks are repaired through mechanisms including homologous recombination and non-homologous end-joining pathways [30,31]. Therefore, the case-control differences we observed in the two repair phases may be a result

of differences in the predominance of one type of damage versus the other, in the two phases, and subsequent differential requirements for repair mechanisms to be activated. A valid criticism of our methods is that we investigated initial and residual damage after 45 min of exposure to 9 Gy irradiation. Our 'repair assay' evaluated residual damage in the comet tail. We cannot exclude other causes of the decrease in DNA tail, including mis-joining of the strands or apoptosis, without additional independent tests. Allowing recovery time >45 min may reveal additional information regarding the potential differential DNA damage and repair profile of prostate cancer cases and controls.

In agreement with our previous observation [14], our analysis among control subjects showed that DNA repair was slightly higher during the first 15 min in smokers compared to never/former smokers, although the baseline damage was not affected by smoking status. Data from other studies have suggested that current and/or heavier smokers have more proficient DNA repair than former/never or lighter smokers and an adaptive response to tobacco carcinogens such that leads to up-regulation of DNA repair capacity in response to chronic tobacco-related insults has been proposed [32,33]. Moreover, significant differences in DNA damage between smokers and non-smokers were measured by the comet assay under baseline culture conditions in whole blood cells [34]; however, we were unable to reproduce these findings, because the electrophoretic conditions of the comet assay protocol that we followed were adjusted to minimize baseline DNA damage, to evaluate the repair kinetics; therefore, damage due to environmental exposures could not be assessed.

Our analysis among control subjects also showed that being overweight was associated with increased DNA damage at the 15 min time point following irradiation. Results by Lockett and colleagues [20] showed a marginally significant interaction between BMI and basal damage in prostate cancer risk. The role of BMI in cancer risk has been under investigation and although studies have found a slightly increased risk with higher BMI [35,36], recent data suggest that obesity may be more consistently related to aggressive prostate tumors [1].

In our analysis, we failed to see a decline of DNA repair with advancing age, most probably due to the age distribution of our population and the limited sample size. The exponential increase of prostate cancer associated with aging may reflect the accumulation of DNA damage as a result of a series of processes including oxidative stress, loss of antioxidant defense mechanisms, inflammation, environmental carcinogens, or decrease in DNA repair capacity [37,38].

Our study has several limitations and the results need to be interpreted with caution. We had limited sample size and thus limited power to detect an association. The disappearance of the comet tail is only a rough approximation of the repair process and does not evaluate in detail any of the relevant enzymatic processes. In addition, the phenotype may be influenced by factors indirectly linked to DNA repair including chromatin structure [39]. The comet assay measures rejoining of strand breaks, which are not necessarily joined correctly: in other words, the repair measure would include mis-repair. It is known that mis-repaired lesions lead to biological effects, such as chromosomal aberrations, which could lead to genetic instability, transformation, and cancer [40]. The monitoring of repair in the population would ideally be done on the target tissue. However, this is not practical, and whole blood cultures have been used as a surrogate. Moreover, there is the possibility of 'reverse causation'; case status might be a potential confounder, and it would be more informative to show the effect of DNA damage and repair in a prospective study. However, there is strong evidence that the repair phenotypes follow a heritable trait and that repair of gamma radiation damage in particular has high heritability [41].

Our data suggest that DNA repair kinetics following irradiation differ slightly between prostate cancer cases and controls. While prostate cancer cases were less efficient at repairing the DNA damage at the initial phase following exposure to gamma radiation, their repair kinetic increases in the later phase. The overall damage repaired within 45 min was approximately 65% and was independent of case status. Additional repair assays and mutagens, longer time-points and examination of the target tissue would be beneficial for further elucidation of the role of DNA repair pathways in prostate carcinogenesis.

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Reference List

1. Hsing AW, Chokkalingam AP. Prostate cancer epidemiology. *Front Biosci* 2006;11:1388–1413. [PubMed: 16368524]
2. Gayther SA, de Foy KA, Harrington P, Pharoah P, Dunsmuir WD, Edwards SM, Gillett C, rdern-Jones A, Dearnaley DP, Easton DF, Ford D, Shearer RJ, Kirby RS, Dowe AL, Kelly J, Stratton MR, Ponder BA, Barnes D, Eeles RA. The frequency of germ-line mutations in the breast cancer predisposition genes BRCA1 and BRCA2 in familial prostate cancer. The Cancer Research Campaign/British Prostate Group United Kingdom Familial Prostate Cancer Study Collaborators. *Cancer Res* 2000;60:4513–4518. [PubMed: 10969800]
3. Xu J, Zheng SL, Turner A, Isaacs SD, Wiley KE, Hawkins GA, Chang BL, Bleecker ER, Walsh PC, Meyers DA, Isaacs WB. Associations between hOGG1 sequence variants and prostate cancer susceptibility. *Cancer Res* 2002;62:2253–2257. [PubMed: 11956079]
4. van Gils CH, Bostick RM, Stern MC, Taylor JA. Differences in base excision repair capacity may modulate the effect of dietary antioxidant intake on prostate cancer risk: an example of polymorphisms in the XRCC1 gene. *Cancer Epidemiol. Biomarkers Prev* 2002;11:1279–1284. [PubMed: 12433703]
5. Dong X, Wang L, Taniguchi K, Wang X, Cunningham JM, McDonnell SK, Qian C, Marks AF, Slager SL, Peterson BJ, Smith DI, Cheville JC, Blute ML, Jacobsen SJ, Schaid DJ, Tindall DJ, Thibodeau SN, Liu W. Mutations in CHEK2 associated with prostate cancer risk. *Am. J. Hum. Genet* 2003;72:270–280. [PubMed: 12533788]
6. Malins DC, Johnson PM, Wheeler TM, Barker EA, Polissar NL, Vinson MA. Age-related radical-induced DNA damage is linked to prostate cancer. *Cancer Res* 2001;61:6025–6028. [PubMed: 11507046]
7. Velasco A, Albert PS, Rosenberg H, Martinez C, Leach FS. Clinicopathologic implications of hMSH2 gene expression and microsatellite instability in prostate cancer. *Cancer Biol. Ther* 2002;1:362–367. [PubMed: 12432247]
8. Hu JJ, Hall MC, Grossman L, Hedayati M, McCullough DL, Lohman K, Case LD. Deficient nucleotide excision repair capacity enhances human prostate cancer risk. *Cancer Res* 2004;64:1197–1201. [PubMed: 14871857]
9. Angele S, Falconer A, Edwards SM, Dork T, Bremer M, Moullan N, Chapot B, Muir K, Houlston R, Norman AR, Bullock S, Hope Q, Meitz J, Dearnaley D, Dowe A, Southgate C, rdern-Jones A, Easton DF, Eeles RA, Hall J. ATM polymorphisms as risk factors for prostate cancer development. *Br. J. Cancer* 2004;91:783–787. [PubMed: 15280931]

10. Ritchey JD, Huang WY, Chokkalingam AP, Gao YT, Deng J, Levine P, Stanczyk FZ, Hsing AW. Genetic variants of DNA repair genes and prostate cancer: a population-based study. *Cancer Epidemiol. Biomarkers Prev* 2005;14:1703–1709. [PubMed: 16030105]
11. Berwick M, Vineis P. Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J. Natl. Cancer Inst* 2000;92:874–897. [PubMed: 10841823]
12. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res* 1988;175:184–191. [PubMed: 3345800]
13. Collins AR. Investigating oxidative DNA damage and its repair using the comet assay. *Mutat. Res* 2009;681:24–32. [PubMed: 18054270]
14. Saha DT, Davidson BJ, Wang A, Pollock AJ, Orden RA, Goldman R. Quantification of DNA repair capacity in whole blood of patients with head and neck cancer and healthy donors by comet assay. *Mutat. Res* 2008;650:55–62. [PubMed: 18032094]
15. Schmezer P, Rajaei-Behbahani N, Risch A, Thiel S, Rittgen W, Drings P, Dienemann H, Kayser KW, Schulz V, Bartsch H. Rapid screening assay for mutagen sensitivity and DNA repair capacity in human peripheral blood lymphocytes. *Mutagenesis* 2001;16:25–30. [PubMed: 11139596]
16. Schabath MB, Spitz MR, Grossman HB, Zhang K, Dinney CP, Zheng PJ, Wu X. Genetic instability in bladder cancer assessed by the comet assay. *J. Natl. Cancer Inst* 2003;95:540–547. [PubMed: 12671022]
17. Gontijo AM, Elias FN, Salvadori DM, de Oliveira ML, Correa LA, Goldberg J, Trindade JC, de Camargo JL. Single-cell gel (comet) assay detects primary DNA damage in nonneoplastic urothelial cells of smokers and ex-smokers. *Cancer Epidemiol. Biomarkers Prev* 2001;10:987–993. [PubMed: 11535552]
18. Iwakawa M, Goto M, Noda S, Sagara M, Yamada S, Yamamoto N, Kawakami Y, Matsui Y, Miyazawa Y, Yamazaki H, Tsuji H, Ohno T, Mizoe J, Tsujii H, Imai T. DNA repair capacity measured by high throughput alkaline comet assays in EBV-transformed cell lines and peripheral blood cells from cancer patients and healthy volunteers. *Mutat. Res* 2005;588:1–6. [PubMed: 16260175]
19. Colleu-Durel S, Guitton N, Nourgalieva K, Legue F, Leveque J, Danic B, Chenal C. Alkaline single-cell gel electrophoresis (comet assay): a simple technique to show genomic instability in sporadic breast cancer. *Eur. J. Cancer* 2004;40:445–451. [PubMed: 14746864]
20. Lockett KL, Hall MC, Clark PE, Chuang SC, Robinson B, Lin HY, Su LJ, Hu JJ. DNA damage levels in prostate cancer cases and controls. *Carcinogenesis* 2006;27:1187–1193. [PubMed: 16364923]
21. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ. Mol. Mutagen* 2000;35:206–221. [PubMed: 10737956]
22. Burlinson B, Tice RR, Speit G, Agurell E, Brendler-Schwaab SY, Collins AR, Escobar P, Honma M, Kumaravel TS, Nakajima M, Sasaki YF, Thybaud V, Uno Y, Vasquez M, Hartmann A. Fourth International Workgroup on Genotoxicity testing: results of the in vivo Comet assay workgroup. *Mutat. Res* 2007;627:31–35. [PubMed: 17118697]
23. Kumaravel TS, Jha AN. Reliable Comet assay measurements for detecting DNA damage induced by ionising radiation and chemicals. *Mutat. Res* 2006;605:7–16. [PubMed: 16621680]
24. Frankenberg-Schwager M. Review of repair kinetics for DNA damage induced in eukaryotic cells in vitro by ionizing radiation. *Radiother. Oncol* 1989;14:307–320. [PubMed: 2657873]
25. Alcaraz A, Hammerer P, Tubaro A, Schroder FH, Castro R. Is There Evidence of a Relationship between Benign Prostatic Hyperplasia and Prostate Cancer? Findings of a Literature Review. *Eur. Urol.* 2008
26. Au WW. Mutagen sensitivity assays in population studies. *Mutat. Res* 2003;544:273–277. [PubMed: 14644328]
27. Itez-Bribiesca L, Sanchez-Suarez P. Oxidative damage, bleomycin, and gamma radiation induce different types of DNA strand breaks in normal lymphocytes and thymocytes. A comet assay study. *Ann. N. Y. Acad. Sci* 1999;887:133–149. [PubMed: 10668470]
28. Christmann M, Tomicic MT, Roos WP, Kaina B. Mechanisms of human DNA repair: an update. *Toxicology* 2003;193:3–34. [PubMed: 14599765]

29. Wood RD, Mitchell M, Sgouros J, Lindahl T. Human DNA repair genes. *Science* 2001;291:1284–1289. [PubMed: 11181991]
30. Pierce AJ, Stark JM, Araujo FD, Moynahan ME, Berwick M, Jasin M. Double-strand breaks and tumorigenesis. *Trends Cell Biol* 2001;11:S52–S59. [PubMed: 11684443]
31. Jackson SP. Sensing and repairing DNA double-strand breaks. *Carcinogenesis* 2002;23:687–696. [PubMed: 12016139]
32. Shen H, Spitz MR, Qiao Y, Guo Z, Wang LE, Bosken CH, Amos CI, Wei Q. Smoking, DNA repair capacity and risk of nonsmall cell lung cancer. *Int. J. Cancer* 2003;107:84–88. [PubMed: 12925960]
33. Wei Q, Cheng L, Amos CI, Wang LE, Guo Z, Hong WK, Spitz MR. Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. *J. Natl. Cancer Inst* 2000;92:1764–1772. [PubMed: 11058619]
34. Ellahuene MF, Perez-Alzola LP, Farfan-Urzuu M, Gonzalez-Hormazabal P, Garay M, Olmedo MI, Last JA. Preliminary evaluation of DNA damage related with the smoking habit measured by the comet assay in whole blood cells. *Cancer Epidemiol. Biomarkers Prev* 2004;13:1223–1229. [PubMed: 15247134]
35. Gronberg H, Damber L, Damber JE. Total food consumption and body mass index in relation to prostate cancer risk: a case-control study in Sweden with prospectively collected exposure data. *J. Urol* 1996;155:969–974. [PubMed: 8583620]
36. Cerhan JR, Torner JC, Lynch CF, Rubenstein LM, Lemke JH, Cohen MB, Lubaroff DM, Wallace RB. Association of smoking, body mass, and physical activity with risk of prostate cancer in the Iowa 65+ Rural Health Study (United States). *Cancer Causes Control* 1997;8:229–238. [PubMed: 9134247]
37. Khandrika L, Kumar B, Koul S, Maroni P, Koul HK. Oxidative stress in prostate cancer. *Cancer Lett* 2009;282:125–136. [PubMed: 19185987]
38. Bostwick DG, Alexander EE, Singh R, Shan A, Qian J, Santella RM, Oberley LW, Yan T, Zhong W, Jiang X, Oberley TD. Antioxidant enzyme expression and reactive oxygen species damage in prostatic intraepithelial neoplasia and cancer. *Cancer* 2000;89:123–134. [PubMed: 10897009]
39. Morrison AJ, Shen X. DNA repair in the context of chromatin. *Cell Cycle* 2005;4:568–571. [PubMed: 15753656]
40. Frankenberg-Schwager M, Frankenberg D. DNA double-strand breaks: their repair and relationship to cell killing in yeast. *Int. J. Radiat. Biol* 1990;58:569–575. [PubMed: 1976718]
41. Wu X, Gu J, Spitz MR. Mutagen sensitivity: a genetic predisposition factor for cancer. *Cancer Res* 2007;67:3493–3495. [PubMed: 17440053]

Table 1

Characteristics of study population

	Cases (N=55)	Healthy controls (N=53)	p ₁ -value	Biopsy controls (N=71)	p ₂ -value	p ₃ -value
<i>Age, y (SD)</i>	65.4 (7.2)	61.2 (8.4)	0.01	65.1 (8.9)	0.85	0.01
<i>Race, N (%)</i>						
Caucasian	40 (73)	51 (96)		38 (54)		
African	14 (25)	2 (4)		30 (42)		
American	1 (2)	0 (0)	0.01	3 (4)	0.10	<0.01
Other						
<i>Smoking Status, N (%)</i>						
Never	21 (48)	9 (18)		28 (47)		
Former	18 (41)	26 (52)		26 (43)		
Current	5 (11)	15 (30)	<0.01	6 (10)	1.00	<0.01
BMI						
<25	21 (57)	26 (53)		18 (37)		
≥25	16 (43)	23 (47)	0.73	31 (63)	0.06	0.10
<i>BPH, N (%)</i>						
Yes	31 (70)	32 (60)		49 (77)		
No	13 (30)	21 (40)	0.30	15 (23)	0.51	0.07
PSA						
≥4	40 (73)	9 (17)		50 (70)		
<4	15 (27)	43 (83)	<0.01	21 (30)	0.78	<0.01
<i>FH prostate cancer, N (%)</i>						
Yes	11 (28)	14 (27)		13 (21)		
No	29 (72)	38 (73)	0.95	48 (79)	0.47	0.48
<i>Household Income</i>						
≥100K	26 (60)	27 (52)		17 (27)		
<100K	17 (40)	25 (48)	0.40	45 (73)	<0.01	<0.01

BMI=body mass index

BPH=benign prostatic hyperplasia

PSA=prostate specific antigen

FH prostate cancer = Family history of prostate cancer

p1=comparison of prostate cancer cases and healthy controls
p2=comparison of prostate cancer cases and biopsy controls
p3=comparison of healthy controls and biopsy controls

Table 2

Effect of prostate cancer status on the DNA repair kinetic

Dose (Gy)-repair (min)	Cases (N=55)		Healthy Controls (N=53)		p ₁ -value	Biopsy Controls (N=71)		p ₂ -value	p ₃ -value
	mean (SD)		mean (SD)			mean (SD)			
0-0	1 (1)		1 (1)		0.14	1 (1)		0.33	0.56
9-0	39 (13)		39 (13)		0.94	38(11)		0.55	0.66
9-15	24(9)		22 (9)		0.48	23 (7)		0.53	0.71
9-45	14 (6)		14 (8)		0.63	14 (6)		0.53	0.88
% repair 0-15	38 (16)		42 (17)		0.10	40 (13)		0.54	0.34
% repair 15-45	27 (12)		22 (19)		0.09	23 (14)		0.13	0.82
% repair 0-45	64 (12)		64 (17)		0.77	62 (14)		0.46	0.44

0-0, baseline damage prior to radiation exposure, 9-0, 9 Gy 0 min of repair; 9-15, 9 Gy 15 min of repair; 9-45, 9 Gy 45 min of repair. All repair time points are corrected for baseline damage. % repair for 0-15 and 15-45 min was calculated as discussed in the Materials and Methods Section.

p1=comparison of prostate cancer cases and healthy controls

p2=comparison of prostate cancer cases and biopsy controls

p3=comparison of healthy controls and biopsy controls

Table 3

DNA repair kinetic by study characteristics in healthy controls

Dose (Gy)>repair (min)	N	9-0	9-15	9-45	% repair 0-15	% repair 15-45	% repair 0-45
		mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)
Age categories							
<60	19	41 (13)	24 (10)	14 (7)	43 (16)	23 (10)	65 (12)
≥60	34	38 (13)	22 (8)	14 (8)	42 (17)	22 (22)	63 (19)
<i>p value</i>		0.46	0.38	0.66	0.85	0.45	0.83
Smoking Status							
Never/Former	35	38 (13)	22 (9)	15 (9)	39 (17)	22 (22)	62 (19)
Current	15	46 (9)	23 (8)	14 (5)	49 (12)	21 (8)	70 (8)
<i>p value</i>		0.04	0.65	0.91	0.04	0.59	0.05
BMI							
<25	26	38 (15)	20 (9)	14 (9)	44 (19)	20 (25)	64 (21)
≥25	23	43 (9)	26 (7)	15 (6)	40 (12)	23 (9)	64 (12)
<i>p value</i>		0.37	0.04	0.19	0.25	0.29	0.36
PSA categories							
≥4	9	36 (14)	21 (9)	12 (6)	12 (6)	25 (9)	67 (10)
<4	43	40 (13)	23 (9)	15 (8)	15 (8)	21 (2)	63 (18)
<i>p value</i>		0.26	0.42	0.28	0.60	0.67	0.78
BPH							
Yes	32	37 (13)	21 (8)	14 (8)	41 (17)	22 (23)	62 (19)
No	21	43 (13)	24 (9)	15 (6)	44 (14)	23 (9)	67 (11)
<i>p value</i>		0.07	0.19	0.40	0.88	0.91	0.33
FH prostate cancer							
Yes	14	41 (15)	24 (9)	14 (6)	37 (23)	28 (17)	65 (11)
No	38	38 (13)	22 (9)	14 (8)	43 (12)	20 (19)	63 (18)
<i>p value</i>		0.27	0.48	0.90	0.76	0.48	0.56

Legend is the same as described in Table 2.

BMI=Body Mass Index

PSA=prostate specific antigen

BPH=benign prostatic hyperplasia
FH prostate cancer = Family history of prostate cancer

Table 4

DNA damage and prostate cancer risk

Dose (Gy)-repair (min)	Cases/Combined Controls	OR (95% CI)*	<i>p trend</i>
<i>9-0</i>			
Hyposensitive (<31.3)	13/31	1.00	
Sensitive (31.4-47.3)	25/64	1.00 (0.32-3.11)	
Hypersensitive (>47.4)	17/29	1.71 (0.53-5.51)	0.31
<i>9-15</i>			
Hyposensitive (<15.0)	10/20	1.00	
Sensitive (15.1-29.0)	29/75	0.70 (0.20-2.52)	
Hypersensitive (>29.1)	16/29	1.56 (0.43-5.72)	0.26
<i>9-45</i>			
Hyposensitive (<8.1)	10/23	1.00	
Sensitive (8.2-18.2)	33/69	1.21 (0.37-3.96)	
Hypersensitive (>18.3)	12/32	1.25 (0.34-4.52)	0.77
<i>% repair 0-15 min</i>			
Hypo-efficient (<36)	21/37	1.00	
Efficient (37-52)	25/61	0.60 (0.25-1.45)	
Hyper-efficient (>53)	9/26	0.38 (0.10-1.37)	0.11
<i>% repair 15-45 min</i>			
Hypo-efficient (<17)	10/32	1.00	
Efficient (18-28)	23/55	1.66 (0.52-5.34)	
Hyper-efficient (>29)	22/37	3.24 (0.98-10.66)	0.04
<i>% repair 0-45 min</i>			
Hypo-efficient (<57)	15/35	1.00	
Efficient (58-73)	30/69	1.02 (0.39-2.67)	
Hyper-efficient (>74)	10/20	1.69 (0.46-5.56)	0.51

For risk estimations, healthy and biopsy controls were combined

* ORs are adjusted for age, race, presence of BPH, BMI and smoking status