DNA damage levels in prostate cancer cases and controls

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This study used the alkaline Comet assay to evaluate whether basal or H2O2-induced DNA damage is associated with prostate cancer (CaP) risk. Using lymphocyte samples from 158 CaP cases and 128 controls, collected in an ongoing case–control study, our results showed that basal DNA damage did not differ between cases and controls. However, the H2O2-induced DNA damage level was significantly higher in incident cases (mean ± SD; 6.61 ± 4.43, n = 102) than controls (5.30 ± 3.60, n = 128) or prevalent cases (4.47 ± 3.19; n = 56). Incident cases with a positive smoking history had significantly higher H2O2-induced DNA damage than never-smokers (7.57 ± 4.82 versus 4.52 ± 2.40; P < 0.001). Above-median H2O2-induced DNA damage was associated with a 1.61-fold increase in CaP risk [95% confidence interval (CI) = 0.92–2.81]; after adjustment for age, race, benign prostatic hyperplasia (BPH), smoking history and family history (FH). Using the lowest quartile of H2O2-induced DNA damage as the referent group, the adjusted ORs for the 25th, 50th and 75th quartiles were 0.90 (95% CI = 0.39–2.05), 1.06 (95% CI = 0.48–2.35) and 2.05 (95% CI = 0.96–4.37), respectively (P = 0.046, test for linear trend). The association between CaP and DNA damage was modified by age, smoking history, family history and body mass index. Our results suggest that DNA damage may be associated with CaP risk. However, larger case–control and follow-up studies are warranted to further evaluate the potential application of the alkaline Comet assay in CaP risk assessment and prevention.

Introduction

Prostate cancer (CaP) is the most common cancer in American men (1). In 2005, ~232,090 USA men will be diagnosed with CaP, and 30,350 will die from it (1). The well-established risk factors are ethnicity/race and family history (FH). CaP incidence increases with age (1). An accumulation of DNA damage and a decline in DNA repair during aging may lead to CaP development (2). The mutational spectra of the androgen receptor gene and p53 gene in tumor tissue suggest that both endogenous and exogenous carcinogens play critical roles in human prostate carcinogenesis (3–5). Persistent DNA damage and mutations may result from increased exposure to carcinogens and suboptimal DNA repair (6).

Two previous studies suggest that age-related structural changes in the DNA from CaP tissue are likely a result of oxidative damage induced by hydroxyl radicals (2,7). More than 70% of CaP cases are diagnosed in men over age 65 (1). A previous study reported that the ratio of mutagenic 8-hydroxy to non-mutagenic base lesions in prostate DNA increased ~3-fold in men between the ages of 16 and 83 (2). Another study suggests that substantial age-related changes in DNA structure play a role in the development of CaP in older men (7). Although their results must be validated, they speculate that prostatic epithelial cells may be subjected to chronic oxidative stresses, perhaps as a consequence of the high-level expression of oxidizing enzymes and/or exposure to androgen and/or other steroid hormone metabolites (8,9).

Cytogenetic assays, such as chromosomal aberrations, micronuclei and sister chromatid exchanges, have been used to measure cellular DNA damage (10). This study tested single-cell gel electrophoresis, or the Comet assay, which estimates DNA strand breakage and alkali-labile sites by measuring DNA migration from the nucleus in a single cell. Intact DNA does not migrate far from the nucleus, but relaxed, broken DNA does, resulting in images that look like comets with tails that consist of DNA fragments. The assay has been applied to several population-based studies (11–16). One study used the Comet assay to demonstrate that smokers with elevated DNA damage in their non-neoplastic urothelial cells may be more susceptible to urinary bladder cancer (17). Patients with cervical dysplasia exhibited higher DNA damage in both cervical epithelial cells and peripheral blood lymphocytes (18). Breast cancer cases and women with FH of breast cancer have elevated levels of basal DNA damage, after treatment with either N-methyl-N-nitrosoguanidine or ionizing radiation, and following repair (19,20). To the best of our knowledge, this study is the first using the Comet assay to evaluate whether DNA damage is associated with CaP risk.

Materials and methods

Study population

CaP cases and controls were recruited from the Departments of Urology and Internal Medicine of the Wake Forest University School of Medicine using sequential patient populations as described previously (21). All study subjects received a detailed description of the study protocol and signed their informed consent. In 2005, ~232,090 USA men will be diagnosed with CaP, and 30,350 will die from it (1). The well-established risk factors are ethnicity/race and family history (FH). CaP incidence increases with age (1). An accumulation of DNA damage and a decline in DNA repair during aging may lead to CaP development (2). The mutational spectra of the androgen receptor gene and p53 gene in tumor tissue suggest that both endogenous and exogenous carcinogens play critical roles in human prostate carcinogenesis (3–5). Persistent DNA damage and mutations may result from increased exposure to carcinogens and suboptimal DNA repair (6).

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Lymphocytes were resuspended at a final concentration of 3 × 10^6 cells/ml in RPMI 1640 without phenol red; 50 μl l of lysis solution (Trevigen) for 1 h. After lysis, slides were treated with 0.37 mM of antifade solution (0.37 mM 4,6-diamidino-2-phenylindole dihydrochloride/0.04% propidium iodide/0.04% polyvinyl alcohol) and stained with 1:10 000 SYBR Green in TE buffer, followed by the addition of 4,6-diamidino-2-phenylindole dihydrochloride/0.04% propidium iodide/0.04% polyvinyl alcohol. Cell pellets were resuspended and incubated for 10 min in ice-cold phosphate-buffered saline (PBS) without calcium and magnesium (basal) or containing H2O2 (final concentration of 500 μM). Lymphocytes were resuspended at a final concentration of 3 × 10^6 cells/ml in RPMI 1640 without phenol red; 50 μl of cell suspension (with or without H2O2 treatment) was mixed with 500 μl of low-melting-point agarose (0.5% low-melting-point agarose in PBS), and 50 μl were spread onto two Comet slide wells (Trevigen, Gaithersburg, MD). Slides were allowed to solidify at 4°C for 30 min and then placed in a prechilled lysis solution (Trevigen) for 1 h. After lysis, slides were treated with an alkali solution (300 mM NaOH and 1 mM EDTA) at room temperature for 1 h and electrophoresed in TBE at 200 mA for 1 h and neutralized with 10 mM Tris-HCl (pH 7.5) for 20 min. The alkaline Comet assay was modified from a previously published protocol (20,22). As part of the quality control effort, aliquots of cryopreserved lymphocytes from a healthy individual were used as an internal control for each batch of assay samples. To adjust for anticipated batch-to-batch assay variations, each batch of encoded sample consisted of 50% from CaP cases and 50% from controls. These samples were thawed, suspended slowly in thawing medium (50% fetal bovine serum, 40% RPMI 1640 and 10% dextrose), and centrifuged at 300 g for 4°C for 10 min. Using trypan blue exclusion, we selected samples with viability >90% for the Comet assay. Cell pellets were resuspended and incubated for 10 min in ice-cold phosphate-buffered saline (PBS) without calcium and magnesium (basal) or containing H2O2 (final concentration of 500 μM). Lymphocytes were resuspended at a final concentration of 3 × 10^6 cells/ml in RPMI 1640 without phenol red; 50 μl of cell suspension (with or without H2O2 treatment) was mixed with 500 μl of low-melting-point agarose (0.5% low-melting-point agarose in PBS), and 50 μl were spread onto two Comet slide wells (Trevigen, Gaithersburg, MD). Slides were allowed to solidify at 4°C for 30 min and then placed in a prechilled lysis solution (Trevigen) for 1 h. After lysis, slides were treated with an alkali solution (300 mM NaOH and 1 mM EDTA) at room temperature for 1 h and electrophoresed in TBE at ~0.8 V/cm for 15 min. Slides were fixed in 100% ethanol for 5 min and stored at 4°C until image analysis. Cellular DNA was stained with 1:10 000 SYBR Green in TE buffer, followed by the addition of antifade solution (0.37 mM 4,6-diamidino-2-phenylindole dihydrochloride/90% glycerol in PBS, CaCl2, MgCl2 free) and a coverslip. The slides were analyzed using the LAI Comet Assay Analysis System (Loats Associates, Westminster, MD), and the results presented as the mean comet-tail moment of 50 cells. The comet-tail moment is defined as the product of the fraction of cellular DNA in the comet tail and the tail length. A higher comet-tail moment value represents a greater number of cellular DNA strand breaks.

Statistical analysis
The characteristics of CaP cases and controls were compared using Student’s t-test, χ²-test or Fisher’s exact test. Analysis of covariance was used to compare differences in mean comet-tail moments between cases and controls, after adjusting for characteristics or two-way interactions between case–control status and characteristics. Logistic regression was used to calculate crude and adjusted odds ratios (OR) and 95% confidence intervals (CI). Likelihood ratio test (LRT) was used to test for interactions. All statistical analyses were carried out using the Statistical Analysis System (SAS Institute, Cary, NC) for personal computers and the S-Plus Statistical Package (Insightful Corporation, Seattle, WA).

Results
Table I summarizes study subjects’ demographic characteristics. Age, race, smoking history, FH and body mass index (BMI) did not differ significantly between cases and controls. However, smoking status differed significantly between cases and controls, with a higher percentage of cases being current smokers than controls (18% versus 7%). The proportion of subjects with a history of BPH also differed significantly, with a higher percentage of controls having a history of BPH than did cases (56% versus 42%; P = 0.05).

As part of the laboratory assay quality-control effort, we included a cryopreserved aliquot of lymphocytes from a healthy subject with each batch of assay samples to monitor batch-to-batch assay variability. In 24 batches, the mean ± SD of basal and H2O2-induced comet-tail moments were 0.44 ± 0.15 and 2.44 ± 0.74, respectively, and the coefficient of variation was 35% for basal and 30% for H2O2-induced DNA damage. We also plotted the mean basal and H2O2-induced comet-tail moments of cases and controls separately by assay batch and did not observe any unusual time-dependent drift (data not shown). Overall, our technical variability supports the use of the Comet assay in molecular epidemiology studies.

We first evaluated whether DNA damage differed between the two groups of cases (Table II). For 102 newly diagnosed cases before any treatment, basal and H2O2-induced damage levels were 1.31 ± 1.22 and 6.61 ± 4.43, respectively. For 56 cases diagnosed within 5 years and free of cancer or treatments for at least 6 months prior to study entry, basal and H2O2-induced damage levels were 1.03 ± 0.97 and 4.47 ± 3.19, respectively. No significant difference was evident for basal damage levels between these two groups (P = 0.14). However, mean H2O2-induced damage levels differed significantly between them (P < 0.01). Therefore, data from...
incident cases were used for subsequent case–control comparisons.

In comparing incident cases and controls, there was no overall difference in basal DNA damage between the two groups or after stratifying by age, race, smoking history, smoking status, FH, BPH history and BMI. The incident cases had higher mean H2O2-induced DNA damage compared with controls (6.61 ± 4.43 versus 5.30 ± 3.60; \( P = 0.01 \)) (Table II). Within the incident case group, subjects with a positive smoking history had significantly higher H2O2-induced comet-tail moments compared with never-smokers (7.57 ± 4.82 versus 4.52 ± 2.40; \( P < 0.01 \)). Both former and current smokers had significantly higher H2O2-induced damage than never-smokers (\( P < 0.01 \)). To explore whether dietary behavior change following cancer diagnosis affected the observed lower DNA damage levels in prevalent cases than in incident cases, we pilot-tested the dietary intake and supplementation data using the Block food frequency questionnaire. Prevalent cases (\( n = 40 \)) have significantly higher dietary intakes of antioxidants than incident cases (\( n = 66 \)), including vitamins B1, B6 and C, riboflavin, folate, alpha- and beta-carotene and fruit servings. Furthermore, a higher percentage of prevalent cases than incident cases used supplements, including vitamin A (\( P = 0.07 \)), vitamin D (\( P = 0.06 \)), vitamin E (\( P = 0.09 \)), beta-carotene (\( P = 0.02 \)) and selenium (\( P = 0.01 \)).

In case-only analysis, we also evaluated whether DNA damage was related to tumor aggressiveness, as determined by Gleason score, tumor stage and pretreatment PSA level. Our results showed that in incident cases, neither basal nor H2O2-induced DNA damage levels differed by biopsy Gleason score (\( \leq 7 \) versus \( > 7 \); \( P = 0.26 \) and 0.38). The mean ± SD basal damage value was 1.31 ± 1.26 (\( n = 81 \)) for cases with Gleason score \( \leq 7 \), and 0.90 ± 0.93 (\( n = 13 \)) for cases with Gleason score \( > 7 \). The mean ± SD H2O2-induced damage value was 6.57 ± 4.68 (\( n = 81 \)) and 5.82 ± 2.40 (\( n = 13 \)) for cases with Gleason score \( \leq 7 \) and \( > 7 \), respectively. Similarly, neither basal nor H2O2-induced DNA damage levels differed by tumor stage (\( P = 0.72 \) and 0.67). Neither basal nor H2O2-induced DNA damage levels in controls and incident cases differed by PSA level. In controls, the average basal comet-tail moments for subjects with PSA \( \leq 2 \) and \( > 2–4 \) were 1.13 ± 1.02 and 1.07 ± 0.94, respectively (\( P = 0.81 \)). The average H2O2-induced comet-tail moments were 5.10 ± 3.42 and 5.99 ± 4.31, respectively (\( P = 0.26 \)). In incident cases, the average basal comet-tail moment levels for subjects with PSA \( < 10 \) and \( \geq 10 \) were 1.20 ± 1.28 and 1.39 ± 1.02, respectively (\( P = 0.54 \)). The mean H2O2-induced comet-tail moment levels were 5.99 ± 3.94 and 8.11 ± 5.77, respectively (\( P = 0.13 \)).

When DNA damage level was dichotomized by the median comet-tail moment of controls, there was a slightly higher but non-significant association (OR = 1.20, 95% CI = 0.70–2.06) between basal DNA damage and CaP risk (Table III). When we stratified basal damage values by quartiles of controls, there was no dose-dependent association between DNA damage and CaP risk (\( P = 0.19 \), test for linear trend). However, above-median H2O2-induced DNA damage level was associated with a higher but non-significant 1.61-fold CaP risk (95% CI = 0.92–2.81), after adjusting for age, race, smoking history, FH and BPH. When we stratified by quartiles, there was a significant dose-dependent association between increasing H2O2-induced damage and elevated CaP risk (\( P = 0.046 \), test for linear trend). Using the lowest quartile of DNA damage as the referent group, the adjusted ORs for the 25th, 50th and 75th
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Table III. DNA damage and human prostate cancer risk

<table>
<thead>
<tr>
<th>DNA damage</th>
<th>Control</th>
<th>Incident</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>Basal</td>
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<tr>
<td>Median cut-off</td>
<td></td>
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</tr>
<tr>
<td>Low (≤0.87)</td>
<td>64</td>
<td>48</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>High (&gt;0.87)</td>
<td>64</td>
<td>54</td>
<td>1.13 (0.67–1.89)</td>
<td>1.20 (0.70–2.07)</td>
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<tr>
<td>Quartiles</td>
<td></td>
<td></td>
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<tr>
<td>0.01 to 0.37</td>
<td>32</td>
<td>22</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>0.37 to 0.87</td>
<td>32</td>
<td>26</td>
<td>1.18 (0.56–2.50)</td>
<td>1.29 (0.59–2.82)</td>
</tr>
<tr>
<td>0.87 to 1.47</td>
<td>31</td>
<td>18</td>
<td>0.84 (0.38–1.87)</td>
<td>1.04 (0.46–2.38)</td>
</tr>
<tr>
<td>1.47 to 4.74</td>
<td>36</td>
<td>36</td>
<td>1.59 (0.77–3.26)</td>
<td>1.63 (0.77–3.46)</td>
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<tr>
<td>H2O2-induced</td>
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<tr>
<td>Median cut-off</td>
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<tr>
<td>Low (≤0.471)</td>
<td>64</td>
<td>39</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>High (&gt;0.471)</td>
<td>64</td>
<td>63</td>
<td>1.62 (0.95–2.74)</td>
<td>1.61 (0.92–2.81)</td>
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<tr>
<td>Quartiles</td>
<td></td>
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</tr>
<tr>
<td>0.02 to 2.75</td>
<td>32</td>
<td>21</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>2.75 to 4.71</td>
<td>32</td>
<td>18</td>
<td>0.86 (0.39–1.90)</td>
<td>0.90 (0.39–2.05)</td>
</tr>
<tr>
<td>4.71 to 6.64</td>
<td>32</td>
<td>23</td>
<td>1.10 (0.51–2.36)</td>
<td>1.06 (0.46–2.35)</td>
</tr>
<tr>
<td>6.64 to 17.22</td>
<td>32</td>
<td>40</td>
<td>1.90 (0.93–3.92)</td>
<td>2.05 (0.96–4.37)</td>
</tr>
</tbody>
</table>

*DNA damage measured by comet-tail moment.

**OR adjusted for age, race, smoking history, FH and BPH.

†Values of controls were used as the cut-off.

‡P = 0.046, test for linear trend.

quartiles were 0.90 (95% CI = 0.39–2.05), 1.06 (95% CI = 0.48–2.35) and 2.05 (95% CI = 0.96–4.37), respectively.

We also evaluated whether the association between DNA damage and CaP risk was modified by age, smoking history, FH and BMI. As shown in Table IV, significant interaction was observed between age and level of basal DNA damage (P = 0.047). The association was stronger in the younger age group (OR = 1.82, 95% CI = 0.84–3.95) than older (OR = 0.59, 95% CI = 0.25–1.38) age group. The interaction between basal DNA damage and BMI was marginally significant (P = 0.061). A stronger association between basal damage and CaP risk was observed in subjects with a higher BMI (OR = 2.07; 95% CI = 0.89–4.78) than those with a lower BMI (OR = 0.72; 95% CI = 0.31–1.66). Marginally significant interaction was also observed between H2O2-induced DNA damage and smoking history (P = 0.098). The association between H2O2-induced damage and CaP was observed only in those with a positive smoking history (OR = 2.34; 95% CI = 1.15–4.77), not in non-smokers (OR = 0.71; 95% CI = 0.27–1.92).

Discussion

Increasing evidence suggests the roles of DNA damage/repair in human CaP risk (2,7,8,20–25). Oxidative stress and accumulated genomic damage may contribute to prostate carcinogenesis (9,26). The results from this study showed that the H2O2-induced DNA damage level was significantly higher in incident cases than controls and prevalent cases. However, the mean basal DNA damage level did not differ between cases and controls. There was a dose-dependent association between increasing H2O2-induced DNA damage and higher CaP risk. Our data also demonstrated that the CaP/damage association may be modified by age, smoking history and BMI. In summary, our current results suggest that elevated DNA damage may be associated with human CaP risk.

Although the role of smoking in CaP is unclear, several studies have shown a positive association between smoking and fatal CaP (27–30). Cigarette smoke contains such carcinogens as poly cyclic aromatic hydrocarbons and heterocyclic aromatic amines (31), which can both be activated by prostate cells (32). As shown in Table II, a positive smoking history has a significant effect on DNA damage in incident cases but not in controls. Data from previous studies suggest that current smokers tend to have higher DNA repair capacity compared with former or non-smokers (33), and smokers had higher expression levels of several DNA repair genes than non-smokers (34). Wei et al. (35) showed that heavy smokers have more proficient DNA repair than light smokers. They suggest that current smokers may have an adaptive response to tobacco carcinogens, upregulating their DNA repair in response to chronic tobacco-related insults (36,37). In theory, different 'adaptive responses' or 'damage-induced responses' to chronic exposure may result from genetic variations in drug metabolism and/or DNA repair. The evolving hypothesis is that subjects with lower cancer risk may have up-regulated detoxification and DNA repair enzymes in response to chronic exposures, and subjects with higher cancer risk lack this defense mechanism. Alternatively, extremely high exposures may overwhelm or inhibit the system. To support this hypothesis, our results in Table IV suggest that the association between H2O2-induced damage and CaP is stronger in subjects with a positive smoking history than non-smokers, which suggests that H2O2-induced damage may serve as a susceptibility risk marker, particularly in smokers.

The role of BMI in CaP risk is under intensive investigation. However, the results from a number of previous studies were inconsistent. Some showed no association between CaP and BMI (38–40); others found a slightly increased risk with higher BMI (41–44). One interesting study even showed that men with higher BMI (≥30 kg/m2) had lower CaP risk than men with lower BMI (23–24.9 kg/m2) (45). In addition, one study found that obese men (BMI ≥ 30) had a significantly higher CaP mortality rate than non-obese men (BMI < 25) (46). Our current results showed a marginally significant interaction between BMI and basal damage in CaP risk (Table IV), suggesting that basal DNA damage may serve a CaP risk marker in subjects with high BMI. Intriguingly, a similar interaction was observed in a previous study of breast cancer (20). Future larger studies are warranted to test whether combining BMI and DNA damage data will refine the risk model for CaP development and/or progression.

Although incident cases are usually preferred in case-control studies, DNA damage measurements in lymphocytes may be modulated by tumor-associated factors, such as tumor-associated antigens and cytokines. Therefore, we also evaluated DNA damage levels in samples collected from cancer-free subjects who were diagnosed previously with CaP. To avoid potential survival bias and treatment effects, we limited our recruitment to cases diagnosed with CaP within 5 years and free of treatments and disease for at least 6 months before study entry. Our results showed that basal DNA damage levels were similar in prevalent and incident cases. However, the H2O2-induced DNA damage level was significantly higher in incident cases. We considered two possible explanations: (i) prevalent cases may have changed to healthier dietary habits and/or lifestyles; and/or (ii) tumor-associated factors may be absent in prevalent cases. Regarding the first hypothesis, our preliminary data provided some evidence that...
prevalent cases had significantly higher intakes of dietary antioxidants and supplements than incident cases. Although these data only indirectly support our first hypothesis, they have potential implications in CaP prevention, based on previous results showing that dietary micronutrients may modulate DNA damage and repair (47–54). Previous studies using the Comet DNA damage assay as the biomarker have shown late DNA damage and repair (47–54). Previous studies using the Comet DNA damage assay as the biomarker have shown late DNA damage and repair (47–54). Previous studies using the Comet DNA damage assay as the biomarker have shown late DNA damage and repair (47–54). Previous studies using the Comet DNA damage assay as the biomarker have shown late DNA damage and repair (47–54). Previous studies using the Comet DNA damage assay as the biomarker have shown late DNA damage and repair (47–54). Previous studies using the Comet DNA damage assay as the biomarker have shown late DNA damage and repair (47–54).

We also considered the second hypothesis that tumor-associated factors may modulate H$_2$O$_2$-induced DNA damage. For example, two related tumor-associated factors, transforming growth factor beta and interleukin-6, are targeted because both play roles in DNA damage/repair in addition to their other functions (55,56), and their plasma levels decrease 6–8 weeks after prostatectomy (57). Removing these tumor-associated factors may lower DNA damage levels in prevalent cases in addition to dietary/lifestyle change. Therefore, whether higher DNA damage in incident cases reflects CaP susceptibility and/or tumor status is unclear. In summary, higher dietary antioxidant intakes and/or removal of tumor-associated factors may contribute to lower DNA damage in prevalent cases. Future follow-up studies are warranted to further examine these two possibilities and the potential application of the Comet assay in CaP susceptibility and prevention.

The strengths of this study include a well-controlled sample processing procedure (blood was processed within 2 h after phlebotomy) and storage (cryopreserved lymphocytes had >90–95% viability), extensive laboratory quality-control programs, and objective comet image analysis. Considering its limitation, peripheral lymphocytes are most often used, because they are easily obtained from blood, but whether they reflect DNA damage/repair in the target tissue must be evaluated. One study reported that comet-tail lengths in peripheral lymphocytes are most often used, because they are easily obtained from blood, but whether they reflect DNA damage/repair in the target tissue must be evaluated. One study reported that comet-tail lengths in peripheral lymphocytes are most often used, because they are easily obtained from blood, but whether they reflect DNA damage/repair in the target tissue must be evaluated. One study reported that comet-tail lengths in peripheral lymphocytes are most often used, because they are easily obtained from blood, but whether they reflect DNA damage/repair in the target tissue must be evaluated. One study reported that comet-tail lengths in peripheral lymphocytes are most often used, because they are easily obtained from blood, but whether they reflect DNA damage/repair in the target tissue must be evaluated. One study reported that comet-tail lengths in peripheral lymphocytes are most often used, because they are easily obtained from blood, but whether they reflect DNA damage/repair in the target tissue must be evaluated. One study reported that comet-tail lengths in peripheral lymphocytes are most often used, because they are easily obtained from blood, but whether they reflect DNA damage/repair in the target tissue must be evaluated. One study reported that comet-tail lengths in peripheral lymphocytes are most often used, because they are easily obtained from blood, but whether they reflect DNA damage/repair in the target tissue must be evaluated. One study reported that comet-tail lengths in peripheral lymphocytes are most often used, because they are easily obtained from blood, but whether they reflect DNA damage/repair in the target tissue must be evaluated.

We also considered the second hypothesis that tumor-associated factors may modulate H$_2$O$_2$-induced DNA damage. For example, two related tumor-associated factors, transforming growth factor beta and interleukin-6, are targeted because both play roles in DNA damage/repair in addition to their other functions (55,56), and their plasma levels decrease 6–8 weeks after prostatectomy (57). Removing these tumor-associated factors may lower DNA damage levels in prevalent cases in addition to dietary/lifestyle change. Therefore, whether higher DNA damage in incident cases reflects CaP susceptibility and/or tumor status is unclear. In summary, higher dietary antioxidant intakes and/or removal of tumor-associated factors may contribute to lower DNA damage in prevalent cases. Future follow-up studies are warranted to further examine these two possibilities and the potential application of the Comet assay in CaP susceptibility and prevention.

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warranted to further test the potential application of the alkaline Comet assay in CaP risk assessment and prevention.

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Conflict of Interest Statement: None declared.

References


DNA damage levels in prostate cancer


