Deficient Nucleotide Excision Repair Capacity Enhances Human Prostate Cancer Risk

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ABSTRACT

Prostate cancer (CaP) is the most commonly diagnosed non-skin cancer and the second leading cause of cancer death in American men. The etiology of CaP is not fully understood. Because most of the DNA adducts generated by some CaP-related carcinogens, including polycyclic aromatic hydrocarbons, heterocyclic amines, and pesticides, are removed by the nucleotide excision repair (NER) pathway, we pilot tested the hypothesis that CaP is associated with deficient NER capacity (NERC), measured by a plasmid-based host reactivation assay. Using cryopreserved lymphocytes collected in an ongoing, clinic-based case-control study, our results showed that the mean NERC was significantly lower (P = 0.03) in 140 cases (mean ± SD, 8.06 ± 5.17) than in 96 controls (9.64 ± 5.49). There was a significant association between below-median NERC and CaP risk: odds ratio (OR), 2.14; 95% confidence interval (CI), 1.19–3.86, after adjustment for age, race/ethnicity, smoking history, benign prostatic hyperplasia, and family history. This association was stronger in younger (<60 years of age) subjects (OR, 3.98; 95% CI, 1.13–14.02) compared with older (≥60) subjects (OR, 1.74; 95% CI, 0.90–3.37). When we stratified NERC values by quartiles of controls, there was a significant dose-dependent association between lower NERC and elevated CaP risk (P for trend < 0.01). Compared with the highest quartile of NERC as the referent group, the adjusted ORs for the 75th, 50th, and 25th quartiles were: 1.09 (95% CI, 0.46–2.59); 1.81 (95% CI, 0.77–4.27); and 2.63 (95% CI, 1.17–5.95), respectively. This pilot study is the first direct evidence associating deficient NERC with human CaP risk.

INTRODUCTION

In 2003, approximately 220,900 American men will be diagnosed with prostate cancer (CaP), and 28,900 will die from it (1). Its etiology is not fully understood. Ethnicity/race and family history (FH) are associated with CaP risk (1), and incidence increases with age; >75% of all cases are diagnosed in men over 65. An accumulation of genetic abnormalities and a decline in DNA repair during aging may lead to CaP (2), because germline mutations or polymorphisms in DNA repair genes BRCAl/2, CHEK2, XRCCL, and OGG1 are associated with CaP risk (3–6). The relation between smoking and CaP is not clear (7). The majority of the prospective cohort studies found a positive association between current smoking and CaP, when death from CaP was used as the study outcome (8–11). Suspected occupations related to CaP are farming and the metal and rubber industries; the related agents are pesticides, cadmium, and polycyclic aromatic hydrocarbons (12, 13).

The data on the mutational spectrum of the androgen receptor gene and p53 gene and a high incidence of somatic mutation of mitochondrial DNA in human prostate tumor tissue suggest that both endoge-
such as age, race, weight, and height; (b) medical history and medication use; (c) smoking history; and (d) FH of cancer. Men with at least one first-degree relative (FDR) with prostate cancer were considered to have a positive FH. The response rate for cases and controls was 94 and 83%, respectively.

**Sample Processing and Storage.** Whole blood (20 ml) was processed within 2 h after phlebotomy. It was carefully layered on top of Neutrophil Isolation Medium (Cardinal Associates Inc., Santa Fe, NM) and centrifuged at 400 × g for 45 min. The lymphocyte fraction was separated, washed, cryopreserved in RPMI 1640 with 50% fetal bovine serum and 10% DMSO, and stored at −140°C until assay.

**NERC Assay.** This study used a host-cell reactivation NERC assay with luciferase (LUC) as the reporter gene (31). In brief, quick-thawed lymphocytes with >90% viability from each study subject were incubated with photopheresis-aglutinin for 72 h to activate the T lymphocytes. The plasmid containing the LUC gene (pCMVluc) was irradiated with UV (254 nm) at 0 (control) or 700 J/m² and transfected into activated T lymphocytes using the DEAE-dextran method. The host cellular enzymes repaired the photochemical damage in the plasmid. Expression of the plasmid-encoded reporter, LUC, was measured 40 h later using the substrate obtained from Promega Corp. (Madison, WI) with a TD-20/20 Luminometer (Turner Designs, Inc., Sunnyvale, CA). Each sample was assayed in duplicate, and the %NERC activity was expressed as: (LUC expression of damage/zero dose) × 100%. Samples from both cases and controls were processed and assayed concurrently. A list of sample IDs with mixed samples from both cases and controls was given to laboratory personnel for conducting the NERC assay; they were blinded from the case/control status of any individual sample.

**Statistical Analysis.** The distributions of demographic characteristics and potential CaP risk factors were compared between cases and controls using χ². Fisher’s exact, and Student’s t tests. NERC was analyzed as a continuous variable, and linear regression was used to compare the mean difference between cases and controls, unadjusted and adjusted for subject characteristics. We also used the median NERC value of controls as the cutoff to calculate odds ratios (ORs) and 95% confidence intervals (CIs) in evaluating the association between low NERC and CaP risk. Values greater than or equal to the median value of controls were considered to have high NERC, and values less than the median were considered to have low NERC. Quartiles of NERC values of controls were also used as cut points to evaluate the dose-response association. Logistic regression was used to calculate crude and adjusted ORs and 95% CIs. All statistical tests were two-sided. Adjusted ORs were calculated by fitting logistic regression models and adjusting for potential confounders, such as age, race/ethnicity, smoking history, benign prostatic hyperplasia (BPH), and FH. The interactions of NERC with age, race/ethnicity, smoking history, BPH, and FH were assessed by including cross-product terms in the logistic regression. All of the statistical analyses were carried out using the Statistical Analysis System for personal computers (SAS Institute, Cary, NC).

**RESULTS**

Study population characteristics are summarized in Table 1. The mean age was not significantly different (P = 0.32) between controls and CaP cases. The distributions of race, smoking history, smoking status, and FH did not differ significantly between cases and controls. However, the proportion of subjects with BPH differed significantly (P = 0.0001). A higher percentage of controls had a history of BPH (78%) than cases (51%).

As part of the laboratory assay quality control effort, we monitored batch-to-batch assay variability. With each batch of samples, we included a cryopreserved aliquot of lymphocytes from a healthy subject and three Epstein-Barr virus-immortalized human lymphoblastoid cell lines obtained from Coriell Cell Repositories (Camden, NJ): (a) GM00131 was derived from a 23-year-old healthy female Caucasian; (b) GM00892 was from a 12-year-old female Caucasian with chromosomal abnormalities; and (c) GM02246 was from a 30-year-old female Caucasian with xeroderma pigmentosum complementation group C. During a 16-month assay period, the mean ± SD was 4.3 ± 1.35 (n = 9) for cryopreserved aliquots of lymphocytes from a healthy volunteer; the batch-to-batch assay coefficient of variation (CV) was 31%. The variation of three lymphoblastoid cell lines ranged from 13 to 30%. The mean ± SD was 15.48 ± 2.00 for GM00131 (n = 13; CV, 13%), 25.62 ± 7.35 for GM00892 (n = 6; CV, 29%), and 2.53 ± 0.75 for GM02246 (n = 13; CV, 30%), respectively. We also plotted the mean NERC of cases and controls separately by assay batch and did not observe any unusual time-dependent drift (data not shown). Overall, our technical variability was acceptable to support the use of the NERC assay in molecular epidemiological studies.

To evaluate whether DNA repair is affected by the presence of tumors, we first compared the NERC values of two groups of cases. For newly diagnosed cases before any treatment, the mean ± SD was 8.22 ± 5.24 (n = 75). For cases diagnosed within 5 years and free of cancer/treatments for at least 6 months before study entry, the mean ± SD of NERC was 7.87 ± 5.12 (n = 65). No significant difference was evident between these two groups of cases (P = 0.69). We also evaluated whether NERC was related to tumor stage or histological grade as quantified by Gleason score. Our results showed that NERC values did not differ by tumor grade (P = 0.93). The mean ± SD was 8.07 ± 5.41 (n = 93) and 8.16 ± 4.77 (n = 43) for cases with Gleason score <7 and ≥7, respectively. Therefore, the combined data for cases were used for all of the subsequent analyses.

CaP patients had significantly lower mean NERC than controls (P = 0.02) (Table 2). Age, race, smoking status, BPH, and FH did not affect NERC after adjustment for case/control status. Interestingly, our data showed that younger cases (<60 years of age) had lower NERC compared with older cases (>60). In addition, cases with below-median NERC (of controls) were diagnosed at a marginally significant younger age (mean ± SD, 64.76 ± 8.99, n = 90) compared with cases with above-median NERC (67.57 ± 7.10, n = 50; P = 0.06).

When NERC values were dichotomized by the median NERC value of controls, below-median NERC was associated with an elevated CaP risk (Table 3). After adjustment for age, race, smoking history, BPH, and FH, the OR was 2.14 (95% CI, 1.39–3.29). The stratified NERC values by quartiles of controls, there was a significant dose-dependent association between lower NERC and elevated CaP risk (P (test for linear trend) < 0.0001). Compared with the highest quartile of NERC as the referent group, the adjusted ORs for the 75th, 50th, and 25th quartiles were: 1.09 (95% CI, 0.46–2.59), 1.81 (95% CI, 0.77–4.27), and 2.63 (95% CI, 1.17–5.95), respectively. In stratified anal-
DISCUSSION

Our current findings support the hypothesis that deficient NERC, measured by a plasmid-based host reactivation assay, is associated with CaP risk. This observation is supported by several lines of evidence: (a) most prospective cohort studies have found a positive association between recent smoking and CaP risk (8–11), and many of the DNA adducts resulting from tobacco-related carcinogens are repaired by NER; (b) NER plays a critical role in repairing DNA damage induced by suspected CaP carcinogens related to various occupations (12); and (c) prostate cells can activate two classes of chemical carcinogens, polycyclic aromatic hydrocarbons and heterocyclic amines (32); bulky DNA adducts derived from both classes of carcinogens are repaired by NER; (33, 34).

The strength of this study includes its high response rate (94% for cases and 83% for controls), high-quality sample processing procedure (blood was processed within 2 h after phlebotomy) and storage (cryopreserved lymphocytes had greater than 90–95% viability), and extensive quality-control programs for laboratory assays and database management. However, we must also consider its limitations. Because it is clinic based, the presence of other medical conditions may have influenced NERC and case/control comparisons. For example, our data in Table 2 suggest that a history of BPH may be associated with lower NERC in both cases and controls. Therefore, we conclude that NERC is not influenced by the presence of CaP and may serve as a susceptibility marker for CaP. Therefore, we are currently recruiting disease-free controls who attend the Internal Medicine and Family Medicine Clinics for routine annual physical examination.

Although case-control studies prefer newly diagnosed, pretreatment cancer cases, we wanted to test whether NERC measurement in lymphocytes might be influenced by tumor-associated factors (e.g., tumor-associated antigens and cytokines). Therefore, we also evaluated NERC in samples collected from cancer-free subjects who were diagnosed previously with CaP. To avoid potential survival bias and treatment effect, 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 NERC was similar in these two groups of cases, and both groups have lower NERC than controls. Therefore, we conclude that NERC is not influenced by the presence of CaP and may serve as a susceptibility marker for CaP.

Another question is related to the variability of the NERC assay. In our study, the variation of three lymphoblastoid cell lines ranged from 13% to 30%. This value is within the range reported by two other laboratories: 27.8%–51.7% (n = 9–12 batches) with two UV dosages and 5.4%–7.2% (n = 4 batches), respectively (29, 31). Assay variability obviously depends mainly on techniques, reagent, and instrumentation. Higher assay variability is expected with larger numbers of batches and a longer experimental period, and it is critical to include quality-control programs for laboratory assays and database management. However, we must also consider its limitations. Because it is clinic based, the presence of other medical conditions may have influenced NERC and case/control comparisons. For example, our data in Table 2 suggest that a history of BPH may be associated with lower NERC in both cases and controls. Therefore, we conclude that NERC is not influenced by the presence of CaP and may serve as a susceptibility marker for CaP.

### Table 2 NERC by selected characteristics of study subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Category</th>
<th>Control (n)</th>
<th>Mean (SD)</th>
<th>Case (n)</th>
<th>Mean (SD)</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>&lt;50</td>
<td>96</td>
<td>9.64 (5.49%)</td>
<td>140</td>
<td>8.06 (5.17%)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>50–59</td>
<td>18</td>
<td>8.85 (5.35%)</td>
<td>35</td>
<td>6.33 (3.26%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>≥70</td>
<td>30</td>
<td>10.31 (5.79%)</td>
<td>48</td>
<td>8.58 (5.09%)</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td>African American</td>
<td>9</td>
<td>11.47 (8.47%)</td>
<td>13</td>
<td>8.46 (7.20%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking Status</td>
<td>Never</td>
<td>29</td>
<td>10.11 (6.18%)</td>
<td>45</td>
<td>7.98 (4.50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking Status</td>
<td>Former</td>
<td>58</td>
<td>9.52 (5.04%)</td>
<td>76</td>
<td>8.38 (5.69%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking Status</td>
<td>Current</td>
<td>9</td>
<td>8.83 (6.37%)</td>
<td>19</td>
<td>6.95 (4.47%)</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>BPH</td>
<td>No</td>
<td>21</td>
<td>10.86 (5.95%)</td>
<td>67</td>
<td>8.53 (5.30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Yes</td>
<td>75</td>
<td>9.29 (5.34%)</td>
<td>71</td>
<td>7.61 (5.11%)</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>FH&lt;sup&gt;c&lt;/sup&gt;</td>
<td>No</td>
<td>80</td>
<td>9.65 (5.44%)</td>
<td>105</td>
<td>7.65 (5.02%)</td>
<td></td>
<td></td>
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<tr>
<td>FH&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Yes</td>
<td>14</td>
<td>8.59 (5.05%)</td>
<td>34</td>
<td>9.49 (5.42%)</td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

a Difference in NERC by selected characteristics adjusted for age/control status.

b Difference in NERC by case/control status adjusted for selected characteristics.

c FH, family history of prostate cancer in first-degree relatives.

### Table 4 Association between NERC and CaP Risk by Age and FH

<table>
<thead>
<tr>
<th>Age/FH</th>
<th>NERC</th>
<th>Control</th>
<th>Case</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)&lt;sup&gt;g&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50%</td>
<td>High (≥8.7%)</td>
<td>11</td>
<td>7</td>
<td>1.00 (Ref.)</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>&lt;50%</td>
<td>Low (&lt;8.7%)</td>
<td>43</td>
<td>37</td>
<td>1.00 (Ref.)</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>≥50%</td>
<td>High (≥8.7%)</td>
<td>33</td>
<td>60</td>
<td>1.56 (0.85–2.88)</td>
<td>1.74 (0.90–3.37)</td>
</tr>
<tr>
<td>≥50%</td>
<td>Low (&lt;8.7%)</td>
<td>61</td>
<td>47</td>
<td>1.00 (Ref.)</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>No FH</td>
<td>High (≥8.7%)</td>
<td>39</td>
<td>35</td>
<td>1.00 (Ref.)</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>No FH</td>
<td>Low (&lt;8.7%)</td>
<td>67</td>
<td>40</td>
<td>1.00 (Ref.)</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>FH</td>
<td>High (≥8.7%)</td>
<td>16</td>
<td>15</td>
<td>1.00 (Ref.)</td>
<td>1.00 (Ref.)</td>
</tr>
<tr>
<td>FH</td>
<td>Low (&lt;8.7%)</td>
<td>7</td>
<td>9</td>
<td>1.27 (0.36–4.41)</td>
<td>1.26 (0.34–4.63)</td>
</tr>
</tbody>
</table>

<sup>g</sup> OR adjusted for race, smoking history, and BPH.

9.29 ± 5.34; 14% decrease). Because the proportion of controls with a BPH history was higher than that of the case group, the true difference in NERC between CaP cases and disease-free controls is probably larger than what is reported here. However, differences may also be exaggerated because of other, unknown factors. To resolve this question, we are currently recruiting disease-free controls who attend the Internal Medicine and Family Medicine Clinics for routine annual physical examination.
between NERC and cancer risk. Last, we also consider whether the repair measurement in lymphocytes may reflect the function in target tissue. Lymphocytes were used as a surrogate in the current study, and their validity for predicting the function of normal prostate cells is under evaluation.

Another interesting observation is that our mean NERC in controls (mean ± SD, 9.64 ± 5.49) is very similar to that reported in two other populations (9.28 ± 4.41 and 10.5 ± 5.1) using either a different damage agent [benzo(a)pyrene-diol-epoxide] or reporter gene (chloroamphicolinic acetyltransferase; Refs. 28, 30). In addition, the case/control difference reported in the current study (16%) is very similar to that of breast cancer (22%), non-small cell lung cancer (16%), and cutaneous malignant melanoma (19%; Refs. 26, 28, 30). These quite consistent findings from different studies suggest that deficient NER may be a common risk factor for different types of human cancers.

Furthermore, a deficient NERC phenotype may be attributable to polymorphic traits and/or exposure and exist at a higher frequency in cancer patients than in cancer-free controls. Unfortunately, the current NERC assay is quite labor intensive and costly and therefore not suitable for population-based screening. Genetic profiling and computational modeling that can predict NERC will have great potential for future human cancer risk assessment. It is critical to evaluate whether NERC can reflect the sum contribution of multiple, functionally significant variants in NER genes that result in a “deficient NERC at-risk” phenotype (35, 36). We are currently testing the functional significance of several amino acid substitution variants of NER genes, including ERCC2 D312N, ERCC2 K751Q, ERCC5 D1103H, ERCC4 R415Q, and XPC K939Q.

Previous studies suggest that NERC may be modulated by aging and oxidative stress (37, 38). However, inconsistent results were reported concerning the effect of aging on NERC (39). Probably because of our narrow age distribution, we did not observe a significant effect of aging on NERC in controls. Intriguingly, the NERC level was lower in younger cases, which suggests that deficient NERC may contribute to early onset of CaP. This hypothesis is further supported by our finding that cases with below-median NERC were diagnosed about 3 years younger than those with above-median NERC (65 years of age versus 68 years of age). Our data in Table 2 suggest that positive FH is related to slightly lower NERC in controls but higher NERC in cases. It seems that NERC may play a more critical role in sporadic CaP, and other risk factors may contribute to FH-related CaP. This concept is further supported by Table 4, where data show that the NERC-CaP association was stronger in subjects without a FH.

In summary, this study provides the first direct evidence that associates deficient NERC with CaP risk. However, our current findings must be validated in larger studies before we can conclude that deficient NER is a predisposition marker for CaP.

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