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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 ($p_{\text{test for linear trend}}$, 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 *BRCA1/2*, *CHEK2*, *XRCC1*, 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 endogenous and exogenous carcinogens play critical roles in human prostate carcinogenesis (14–16). The results from several previous studies suggest that base excision repair and mismatch repair may play roles in CaP susceptibility (2, 3–6, 17–19). However, direct evidence to support the association between deficient DNA repair capacity and CaP risk remains to be established. Because nucleotide excision repair (NER) plays a critical role in repairing DNA damage induced by several suspected human CaP carcinogens, including polycyclic aromatic hydrocarbons, heterocyclic amines, and pesticides, we believe that, in addition to the base excision repair and mismatch repair pathways, NER should also be evaluated for its role in CaP risk.

NER is the most important and complicated repair process, involving the protein products of >30 genes (20). It has broad specificity and recognizes a wide variety of DNA damage. Lehmann (21) proposed a model for NER as follows: (*a*) DNA damage is recognized by the XPA protein in association with RPA; (*b*) TFIIH (with six subunits: XPB, XPD, p62, p44, p34, and TTDA) is recruited; its helicase activities open the structure, allowing (*c*) nucleases, such as ERCC1-XPF and XPG, to cut the DNA on the 5′ and 3′ ends of the damage, respectively; (*d*) the resulting gap is filled by DNA polymerase, accessory protein replication factor C, and proliferating cell nuclear antigen; and (*e*) the DNA is joined by DNA ligase. Mutations and single nucleotide polymorphisms in critical NER genes may contribute to deficient NER capacity (NERC) and human cancer risk (22–25).

Using a plasmid-based, host-reactivation assay, deficient NERC has been associated with risk of basal cell carcinoma, non-small cell lung cancer, breast cancer, and cutaneous malignant melanoma (26–30). However, to the best of our knowledge, no previous report focuses on the association between NERC and human CaP risk. In this study, we pilot tested the hypothesis that deficient NERC is associated with CaP risk, using the first 236 samples collected in an ongoing, clinic-based case-control study.

MATERIALS AND METHODS

Study Population. Both cases and controls were recruited from the Urology Clinic of the Wake Forest University School of Medicine from October 1998 to November 1999. All subjects received a detailed description of the study protocol and signed informed consent forms, as approved by the medical center's Institutional Review Board. Then blood samples were collected from all subjects. The general eligibility criteria were: (*a*) able to comprehend informed consent; and (*b*) without previously diagnosed cancer. The exclusion criteria were: (*a*) autoimmune disease; (*b*) chronic inflammatory conditions; and (*c*) infections within the past 6 weeks. Two groups of cases were recruited: incident, *i.e.*, newly diagnosed, untreated cases ($n = 75$); and prevalent, cases diagnosed with CaP within 5 years and free of cancer/treatments for at least 6 months before study entry ($n = 65$). Controls were frequency-matched to cases (1:1 ratio to incident cases) by age and race. Two groups of controls were recruited: (*a*) men with normal prostate-specific antigen levels and normal digital rectal exam (DRE); and (*b*) men with abnormal prostate-specific antigen or abnormal digital rectal exam but free of CaP based on negative biopsy results. Case-control status was confirmed by prostate-specific antigen level, digital rectal exam results, medical records, and pathology reports. A self-administered questionnaire collected information on: (*a*) demographic factors,

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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 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 phytohemagglutinin for 72 h to activate the T lymphocytes. The plasmid containing the *LUC* gene (pCMV*luc*) was irradiated with UV (254 nm) at 0 (control) or 700 J/m^2 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 χ^2, 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 dosage-dependent 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.

Table 1 *Selected characteristics of study subjects*

	Categories	Control ($n = 96$)	Case ($n = 140$)	P^a
Age	Mean ± SD	64.5 ± 9.7	65.7 ± 8.4	0.32
Age, *n* (%)	<50	8 (8.3)	2 (1.4)	
	50–59	18 (18.8)	35 (25.0)	
	60–69	40 (41.7)	55 (39.3)	
	≥70	30 (31.2)	48 (34.3)	0.05
Race, *n* (%)	African American	9 (9.4)	13 (9.3)	
	Caucasian	87 (90.6)	127 (90.7)	0.98
Smoking history,[b] *n* (%)	No	29 (30.2)	45 (32.1)	
	Yes	67 (69.8)	95 (67.9)	0.75
Smoking status, *n* (%)	Never	29 (30.2)	45 (32.1)	
	Former	58 (60.4)	76 (54.3)	
	Current	9 (9.4)	19 (13.6)	0.52
BPH,[c] *n* (%)	No	21 (21.9)	67 (48.6)	
	Yes	75 (78.1)	71 (51.4)	<0.0001
	Missing	0	2	
FH,[d] *n* (%)	No	80 (85.1)	105 (75.5)	
	Yes	14 (14.9)	34 (24.5)	0.08
	Missing	2	1	

[a] *P*s are from Student's *t* test, Fisher's exact test, or χ^2 test.
[b] Ever smoked 100 cigarettes.
[c] BPH, benign prostatic hyperplasia.
[d] FH, family history of prostate cancer in first-degree relatives.

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, 1.19–3.86). When we stratified NERC values by quartiles of controls, there was a significant dose-dependent association between lower NERC and elevated CaP risk ($p_{\text{test for linear trend}}$, 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. In stratified anal-

Table 2 *NERC by selected characteristics of study subjects*

Characteristics	Category	Control		Case		P^a	P^b
		n	Mean (SD)	*n*	Mean (SD)		
Total		96	9.64% (5.49%)	140	8.06% (5.17%)		0.02
Age	<50	8	10.24% (7.08%)	2	4.90% (3.39%)		
	50–59	18	8.85% (5.35%)	35	6.33% (3.26%)		
	60–69	40	9.37% (5.11%)	55	8.82% (6.01%)		
	≥70	30	10.31% (5.79%)	48	8.58% (5.09%)	0.22	0.02
Race	African American	9	11.47% (8.47%)	13	8.46% (7.20%)		
	Caucasian	87	9.45% (5.12%)	127	8.02% (4.95%)	0.58	0.02
Smoking Status	Never	29	10.11% (6.18%)	45	7.98% (4.50%)		
	Former	58	9.52% (5.04%)	76	8.38% (5.69%)		
	Current	9	8.83% (6.37%)	19	6.95% (4.47%)	0.54	0.02
BPH[c]	No	21	10.86% (5.95%)	67	8.53% (5.30%)		
	Yes	75	9.29% (5.34%)	71	7.61% (5.11%)	0.10	0.01
FH[d]	No	80	9.65% (5.44%)	105	7.65% (5.02%)		
	Yes	14	8.59% (5.05%)	34	9.49% (5.42%)	0.27	0.02

[a] Difference in NERC by selected characteristics adjusted for case/control status.
[b] Difference in NERC by case/control status adjusted for selected characteristics.
[c] BPH, benign prostatic hyperplasia.
[d] FH, family history of prostate cancer in first-degree relatives.

ysis, the association between deficient NERC and CaP risk seemed to be modified by age and FH. As shown in Table 4, the association between low NERC and CaP risk was stronger in the younger group (<60 year of age) compared with that in the older group. In addition, the association was slightly stronger in subjects without a FH.

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 current smoking and CaP mortality (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 primarily repaired by the NER pathway (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 (mean ± SD, 8.53 ± 7.61 *versus* 7.61 ± 5.11; 11% decrease) and controls (10.86 ± 5.95 *versus* 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.

Table 3 *Deficient NERC and human CaP risk*

NERC	Control	Case	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Median cut-off[b]				
High (≥8.7%)	48	50	1.00 (Ref.)	1.00 (Ref.)
Low (<8.7%)	48	90	1.80 (1.06–3.05)	2.14 (1.19–3.86)
Quartiles[b]				
12.10% +	23	26	1.00 (Ref.)	1.00 (Ref.)
8.7% + to 12.10%	25	24	0.85 (0.38–1.88)	1.09 (0.46–2.59)
5.78% + to 8.7%	24	36	1.33 (0.62–2.84)	1.81 (0.77–4.27)
<5.78%	24	54	1.99 (0.95–4.17)	2.63 (1.17–5.95)[c]

[a] Odds ratio adjusted for age, race, smoking history, BPH, and FH.
[b] Values of controls were used as cut-off.
[c] $P = 0.01$, test for linear trend.

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

Age/FH	NERC	Control	Case	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
Age <60	High (≥8.7%)	11	7	1.00 (Ref.)	1.00 (Ref.)
	Low (<8.7%)	15	30	3.14 (1.01–9.75)	3.98 (1.13–14.02)
Age ≥60	High (≥8.7%)	37	43	1.00 (Ref.)	1.00 (Ref.)
	Low (<8.7%)	33	60	1.56 (0.85–2.88)	1.74 (0.90–3.37)
No FH	High (≥8.7%)	39	35	1.00 (Ref.)	1.00 (Ref.)
	Low (<8.7%)	41	70	1.90 (1.05–3.46)	2.44 (1.26–4.71)
FH	High (≥8.7%)	7	15	1.00 (Ref.)	1.00 (Ref.)
	Low (<8.7%)	7	19	1.27 (0.36–4.41)	1.26 (0.34–4.63)

[a] OR adjusted for race, smoking history, and BPH.

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 internal controls and a stringent quality control program. In another important strategy, we also routinely mix samples from both cases and controls in each batch, concluding that although considerable intra-assay variability exists, the variation does not obscure the relationship

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 (chloramphenicol 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 in 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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