

NUCLEOTIDE-EXCISION REPAIR AND PROSTATE CANCER RISK

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Abstract

Prostate cancer (CaP) is the most commonly diagnosed nonskin cancer and the second leading cause of cancer death in American men. Its etiology is not fully understood. Ethnicity/race and family history are associated with it, and incidence increases with age. As with other solid tumors, accumulation of mutations and decline in DNA repair during aging may lead to CaP. However, we believe that conducting a large population screening for every cancer susceptibility gene (e.g., DNA repair) is only meaningful, if we can predict to what extent genetic variants contribute to DNA-repair functional phenotype and CaP risk. This review focuses on the association between CaP and nucleotide excision repair (NER), because some of the DNA adducts generated by CaP-related carcinogens are removed by the NER pathway, and our previous data showed a significant association between lower NER capacity (NERC) and CaP risk. Many laboratories, including ours, have employed a variety of approaches to evaluate the functional significance of DNA-repair, single-nucleotide polymorphisms (SNPs) in human cancer risk assessment. Genetic profiling and computational modeling that can predict NERC may have great potential for CaP-risk assessment, because the current NERC assay is quite labor intensive, costly, and therefore not suitable for population-based screening.

1. Introduction

In 2004, prostate cancer (CaP) accounts for approximately 33% (230,110) of incident cancer cases in American men, and about 29,900 will die from it [1]. While genetic research on human cancer is advancing rapidly, CaP etiology is not fully understood. The only well-established risk factors are race, age, and family history (FH) [1]. African-American men have a higher CaP and mortality rate [1]. More than 70% of cases are diagnosed in men over age 65, possibly explained by the accumulation of mutations and decline in DNA repair during aging [2]. Previous studies in twins and complex segregation analyses suggest that rare autosomal dominant alleles account for a substantial proportion of inherited, early onset cases [3]. Although the relationship between smoking and CaP was not clear [4], most prospective cohort studies found a positive association between current smoking and fatal CaP [5-10]. Farming and the metal and rubber industries were implicated in CaP development [11, 12].

Some of the established CaP-susceptibility genes include *RNASEL*, *ELAC2*, *MSR1*, *AR*, *CYP17*, and *SRD5A2*. In addition to germline mutations, CaP cells contain many somatic mutations, gene deletions and amplifications, chromosomal rearrangements, and changes in DNA methylation [3]. Germline mutations and polymorphisms in DNA-damage response genes, including *BRCA1/2*, *OGG1*, *XRCC1*, *CHEK2*, and *ADPRT*, were associated with CaP risk [13-17]. The implication is that genetic defects in transcription-coupled repair (TCR) associated with *BRCA1*; double-strand break repair and/or homologous recombination related to *BRCA1/2*; cell-cycle checkpoint due to *CHEK2*; and base-excision repair (BER) related to *XRCC1*, *OGG1*, and *ADPRT* may be involved in CaP. The mutational spectrum of the androgen receptor gene, p53 gene, and mitochondria in prostate tumor tissue suggests that both endogenous and exogenous carcinogens play critical roles in human CaP development [18-20]. Therefore, we speculated that multiple DNA-repair pathways play important roles in prostate carcinogenesis. Moreover, we believe that nucleotide-excision repair (NER) is especially important based on two lines of evidence: (a) NER plays a critical role in repairing DNA damage induced by several suspected human CaP carcinogens, including tobacco-related polycyclic aromatic hydrocarbons (PAHs), heterocyclic aromatic amines (HAAs) from well-done meats, and pesticides; and (b) prostate cells can activate PAHs and HAAs [21].

2. The NER Pathway

NER is the most important and complicated repair process, involving the protein products of more than 30-40 genes [22]. It removes the broadest spectrum of genomic damages, including UV-induced photoproducts, bulky mono-adducts, cross-links, and oxidative damage. Several known genetic defects in NER lead to xeroderma pigmentosum, which is associated with a 1000-fold increase in skin cancer as well as a 20-fold increase in other internal tumors [23].

There are two NER subpathways: (a) global-genome repair (GGR), which repairs DNA lesions across the genome; and (b) TCR, which repairs DNA lesions that are specific to the transcribed strand of active genes. These two subpathways differ in the damage-recognition step. In GGR, damage is initially recognized by the XPC-hHR23B complex in association with the XPA-RPA complex as well as the XPE protein [24-27]. XPA-RPA may aid in positioning other repair factors and guiding the nucleases to proper incision sites between single-stranded and duplex DNA [28]. Lesions on the transcribed strand of DNA result in the stalling of RNA polymerase II, which, in TCR, CSA and CSB proteins recognize and remove [29, 30].

In both subpathways, after damage recognition, the TFIIH complex, consisting of two ATP-dependent helicase subunits, ERCC2/XPD (5' to 3') and ERCC3/XPB (3' to 5'), unwind the DNA helix around the lesion, thereby separating the two strands and forming a preincision structure [31, 32]. The endonucleases ERCC5/XPG and ERCC1/XPF then make the 3' and 5' incisions, respectively [33-35]. After removal of the lesion, DNA synthesis is completed by DNA polymerases δ and ϵ with the aid of accessory proteins RFC and PCNA, and the newly

synthesized DNA is then sealed by DNA ligase [36, 37]. Some NER proteins are involved in other cellular processes, such as XPF-ERCC1 in double-strand break repair, RPA, and promotion of other enzymatic activities by XPG with hNTH1 DNA glycosylase [24, 38].

3. NER Capacity (NERC) and Human Cancer Risk

In general, two types of assays have been used to measure NER-GGR activity: (a) unscheduled DNA synthesis, the ability of cells to remove damaged DNA and to synthesize new DNA; and (b) the plasmid-based host-cell reactivation assay, a direct measure of cellular ability to remove lesions from damaged plasmids. The host-cell reactivation assay is preferred, because it uses undamaged cells and measures the entire progression of repair steps leading to the restoration of the biological properties of a reporter gene, rather than just a specific step [39]. In the plasmid-based assay, cells are transfected with either a damaged (or nondamaged, as a control) plasmid harboring a chloramphenicol acetyl transferase (CAT) or luciferase (LUC) gene. Different damaging agents, such as benzo(a)pyrene and UV irradiation, have been used to form bulky DNA adducts, which are repaired mainly by the NER pathway. In theory, the damaging agent produces at least one DNA adduct per plasmid, completely blocking transcription of the CAT or LUC reporter gene without inducing conformational changes in the DNA. A single unrepaired DNA adduct can effectively block CAT or LUC transcription; therefore, any measurable reporter activity will reflect the transfected cells' ability to remove adducts from the plasmid. In general, the cellular % NERC is presented as the ratio of the reporter gene's expression level of the damaged to the undamaged plasmid multiplied by 100%.

The NERC assay has been applied to a number of case-control studies [39-59]: 40% (4/10) (see Table I) showed that skin cancer cases have decreased repair activities (5-58% decrease) compared to normal healthy controls [39-48]. Unfortunately, the sample size is small in most of the earlier. As shown in the two larger studies, skin cancer patients have a 19-42% decreased NERC compared to controls [47, 48]. In 100% (5/5) of lung cancer case-control studies, cancer patients have a significantly decreased NERC (12-55%) compared to controls [49-53]. Two studies that evaluated NERC in head and neck cancer showed conflicting results [54, 55]. However, the larger study showed that head and neck cancer patients had a 31% decrease in NERC compared to controls [54]. The results from three small studies consistently showed that breast cancer cases have a 10-36% lower NERC than controls [56-58]. To the best of our knowledge, our laboratory published the first study evaluating the association between NERC and human CaP risk [59]. This study found that CaP cases have a 20% lower NERC than controls.

In conclusion, various types of human cancer are associated with deficient NERC, which may be attributable to genetic traits and/or exposures that affect cancer patients in higher frequency than study controls.

4. NER Single-Nucleotide Polymorphisms (SNPs)

Highly penetrant prostate cancer genes contribute to a small percentage (5-10%) of CaP, while a high percentage may be related to common polymorphisms in DNA-repair genes [13-17, 60]. Moreover, common genetic polymorphisms and/or exposures may explain the extensive interindividual variability in NERC and the CaP risk that results from lower NERC [59, 61]. A large number of SNPs in different DNA-repair genes have been identified, and some of them have been studied for human cancer susceptibility [62]. However, only few of them have been evaluated in CaP risk. For example, SNPs in a BER gene, *oxoguanine glycosylase 1 (hOGG1)*, were associated with CaP in both sporadic and familial cases [14]. A genetic variant in another BER gene, *X-ray cross-complementation group 1 (XRCC1) R399G*, was associated with elevated CaP risk in individuals with lower vitamin E and lycopene intake [15]. Only one published study has evaluated a NER polymorphism and CaP risk [63]. It demonstrated that combined variant genotypes of *ERCC2/XPD D312N* in NER and *XRCC1 R399G* in BER greatly increased the risk of CaP [63].

To date, more than 40 genes and 200 SNPs have been identified in the NER pathway. In Table II, we summarize the protein functions and the nonsynonymous SNPs (nsSNPs) information of NER genes. With high-throughput genotyping tools increasing the number of NER SNPs identified, we are in a better position to evaluate which SNPs might contribute to altered functional phenotypes and human cancer risk [64].

Our current strategy is first to evaluate two computational algorithms in predicting functional consequences of nsSNPs: Sorting Intolerant from Tolerant (SIFT) [<http://blocks.fhcrc.org/sift/SIFT.html>] and Polymorphism Phenotyping Algorithm (PolyPhen) [<http://www.bork.embl-heidelberg.de/PolyPhen>]. SIFT is based on the hypothesis that important amino acids are conserved; therefore, changes at well-conserved positions will be damaging and affect protein function. A SIFT score less than 0.05 reflects an intolerable change in amino acid. PolyPhen predicts the impact of an amino acid substitution based on its location within the protein structure and the nature of the change. A PolyPhen score higher than 1.5 reflects a possibly damaging change in amino acid. Based on three criteria: (a) variant-allele frequency ≥ 0.05 ; (b) SIFT score < 0.05 ; or (c) PolyPhen score > 1.5 , we also listed targeted NER nsSNP and their reference SNP number (rs#) in Table II for future studies.

5. NER Genotype/Function Relationship

Mutations and SNPs in critical NER genes may contribute to deficient NERC and human cancer risk. The results from a previous study demonstrated that subjects with wild-type genotypes for *ERCC2/XPD K751Q* and *D312N* exhibited the most proficient NERC, while homozygous variant genotypes at either locus showed reduced NERC [51]. The PAT+/+ genotype at *XPC* intron 9 was associated with decreased NERC compared to wild-type genotype carriers [65]. The *ERCC2/XPD 751 Q* allele resulted in a decrease in NERC [66]. This study also showed that subjects with low tanning ability who are carriers of the *ERCC2/XPD 312N* and *751Q* alleles are at increased risk for melanoma [66]. In addition, the presence of the *G* allele at the *XPA* 5' noncoding region, which is located 4 nucleotides upstream from the ATG start codon, was associated with decreased NERC [67].

Using a variant-allele frequency greater than 0.05 as cut-off to gain adequate statistical power, we summarize the preliminary data on the roles of 7 initially targeted nsSNPs in NERC function and CaP risk (Table III). The first 470 controls and 494 prostate cancer cases showed that the *ERCC4/XPF 415 RQ* genotype was associated with a slight but nonsignificant increase in CaP risk (OR=1.37; 95% CI=0.95-1.97), after adjustment for age, benign prostatic hyperplasia, smoking history, and family history. To our surprise, CaP risk was not influenced by the other 6 NER nsSNPs - *ERCC2/XPD D312N*, *ERCC2/XPD K751Q*, *ERCC5 D1104H*, *RAD23B A249V*, *XPC A499V*, and *XPC K939Q* - in univariate analysis. Because many genes involved in the NER pathway are required to maintain genomic integrity, variants in them may have additive/multiplicative effects on NERC function and CaP risk. Therefore, we are currently evaluating additional NER nsSNPs and exploring different computational approaches to assess gene-gene interactions.

As shown in Table III, the SIFT program predicts *ERCC4 R415Q*, *XPC A499V*, and *XPC K939Q* may have functional consequences, while the PolyPhen algorithm predicts the same for *ERCC5 D1104H*. The disagreement could be due to differences in the datasets. Since SIFT focuses on sequence conservation, it may be more sensitive than PolyPhen in identifying the potential impact of variants in highly conserved protein regions. Amino acid changes that were not predicted to be damaging by SIFT could be those that are in linkage disequilibrium with undetermined variants or those which have no known human homologous sequences. Since PolyPhen uses protein structure to make its predictions, it is limited by the amount of protein structural information available.

Using a plasmid-based NERC assay, we identified 4 out of 7 NER nsSNPs with functional implications (e.g., *ERCC2 D312N*, *ERCC2 K751Q*, *ERCC5 D1104H*, and *XPC*

A499V) in Table III [59, preliminary data]. However, only 2 of them (e.g., *ERCC5 D1104H* and *XPC A499V*) were predicted to affect function by either the SIFT or the PolyPhen program. We anticipate that the predicting power will increase as the availability of protein sequencing and structural data increases; therefore, functional predictions by these programs will become more reliable. In summary, computational algorithms may be used to predict the potential functional impact of DNA-repair SNPs, but the predictions should be validated experimentally.

6. Conclusions

The most important problems facing CaP research are identifying high-risk individuals and implementing clinical surveillance, prevention practices, and follow-up care. Germline mutations may be the most important risk factors for hereditary or early-diagnosed CaPs; however, other susceptibility genes and environmental factors may contribute to most CaP cases.

Repair pathways, including NER, play an important role in CaP risk, and genetic variations may contribute to decreased NERC and CaP susceptibility. Although the increased risk associated with individual repair SNPs may be small compared to that conferred by high-penetrant cancer genes, their public health implication may be large, because of their high frequency in the general population.

The SIFT and PolyPhen computational algorithms can be useful in identifying the nsSNPs most likely to affect repair function. However, experiment is crucial in confirming their predictions about functional phenotypes and CaP susceptibility.

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Table I: Application of NERC in Human Cancer Risk Assessment

Site	Reporter/ Damaging Agent	% NERC		% Difference	Reference		
		Cases (n)	Controls (n)				
Skin	CAT	Male	10.1 (14)	Male	9.6 (14)	+5%	[39]
	UV (800J/m ²)	Female	7.2 (24)	Female	9.1 (13)	-26%	
	CAT		7.4 (88)	without FH or actinic keratosis			[40]
	UV (700J/m ²)			8.0 (106)		-9%	
				with FH or actinic keratosis			
				7.3 (29)		-1%	
	CAT	Basal cell carcinoma					[41]
	UV (700J/m ²)	13.0 (76)		12.0 (87)		+8%	
		Squamous cell carcinoma					
		12.2 (25)		11.3 (57)		+7%	
	CAT	# of Basal cell carcinomas					[42]
	UV (700J/m ²)	1	7.8 (55)		7.8 (135)	-1%	
		2	6.8 (15)			-15%	
		3	6.7 (11)			-17%	
		4	6.1 (7)			-28%	
CAT		7.4 (88)		7.8 (135)	-5%*	[43]	
UV (700J/m ²)							
CAT	Females	18.2 (22)		Females	17.4 (40)	+4%	[44]
UV (350 J/m ²)	Males	23.5 (27)		Males	14.1 (28)	+40%	
	Females	8.4 (22)		Females	9.5 (40)	-13%	
UV (700 J/m ²)	Males	16.4 (27)		Males	8.2 (28)	+50%	
CAT	With psoriasis	5.7 (20)		With psoriasis	9.0 (20)	-58%*	[45]
UV (700 J/m ²)	Non-psoriasis	7.1 (20)		Non-psoriasis	6.4 (20)	+10%	
CAT		19.1 (132)			18.4 (145)	+4%	[46]
UV (350 J/m ²)							
LUC		5.0 (280)			8.6 (177)	-42%*	[47]
UV (700 J/m ²)							
CAT		8.5 (312)			10.5 (324)	-19%*	[48]
UV (800J/m ²)							
Lung	CAT		3.3 (51)		5.1 (56)	-55%*	[49]
	BPDE (75 μM)						
	CAT		8.1 (316)		9.8 (316)	-21%*	[50]
	BPDE (60 μM)						
	CAT		7.8 (341)		9.5 (360)	-22%*	[51]
	BPDE (60 μM)						
	CAT		7.8 (467)		9.3 (488)	-16%*	[52]
BPDE (60 μM)							
CAT		7.8 (764)		8.8 (677)	-12%*	[53]	
BPDE (60 μM)							

Head and neck	CAT BPDE (60 μ M)	8.6 (55)	12.4 (61)	-31%*	[54]
	CAT BPDE (60 μ M)	25.1 (9)	26.0 (11)	-4%	[55]
Breast	CAT UV (700J/m ²)	2 Populations 1) 11.2 \pm 3.7 (25) 2) 10.9 \pm 3.6 (16)	2 Populations 1) 13.8 \pm 4.9 (25) 2) 14.2 \pm 5.5 (48)	-19%* -23%*	[56]
	LUC UV (700J/m ²)	5.6 (33)	8.7 (47)	-36%*	[57]
	CAT BPDE (60 μ M)	10.1 (69)	11.1 (79)	-10%*	[58]
	LUC UV (700J/m ²)	8.1 (140)	9.6 (96)	-20%*	[59]
Prostate	LUC UV (700J/m ²)	8.1 (140)	9.6 (96)	-20%*	[59]

P <0.05, difference is statistically significant.

Table II: Nucleotide Excision Repair Genes and Targeted nsSNPs

Gene Symbol	Gene Name	Gene function ¹	# nsSNPs	Targeted nsSNPs ²	rs#
<i>CCNH</i>	Cyclin H	Kinase subunit of TFIIH; involved in transcription by RNA pol II	4	R138K I199M	2266691 1799791
<i>CETN2</i>	Centrin 2/Caltracin 1	Component of the XPC complex; stabilizes XPC in the presence of RAD23B	0	None ³	None
<i>DDB1</i>	Damage specific DNA binding protein 1	Recognition and DNA binding of UV photoproducts and some bulky lesions; interacts with RPA	2	None	None
<i>DDB2</i>	Damage specific DNA binding protein 2	Same as DDB1	3	None	None
<i>ERCC1</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 1	Structure-specific DNA repair endonuclease responsible for the 5' incision	2	P77H A266T	3188420 3212977
<i>ERCC2/ XPD</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 2 (XPD)	5'→3' helicase; member of TFIIH complex; involved in initiating transcription and formation of preincision complex	8	I199M H201Y A270V D312N K751Q	238406 1799792 2266690 1799793 1052559
<i>ERCC3/ XPB</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 3 (XPB)	3'→5' helicase; member of TFIIH complex; involved in initiating transcription and formation of preincision complex	4	G402C	1805162
<i>ERCC4/ XPF</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 4 (XPF)	5'→3' endonuclease; complexes with ERCC1	18	P379S R415Q T576R S662P C745G S747F S768F G875E E912G	1799802 1800067 1800068 2020955 12932917 12928616 12928650 1800124 2020956
<i>ERCC5/ XPG</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 5 (XPG)	3'→5' endonuclease; single-strand specific nuclease; interacts with XPC	26	R181H M254V S311C C529S E627A L670F D1104H	4150295 1047769 2307491 2227869 2227870 1803542 17655
<i>ERCC6/ CSB/ CKN2</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 6 (Cockayne Syndrome B)	Involved in preferential repair of active genes; repair of transcribed strand; interacts with CSA and TFIIH	16	G399D M1097V R1213G R1230P L1308V Q1413R	2228528 2228526 2228527 4253211 2229761 2228529
<i>ERCC8/ CSA/ CKN1</i>	Excision repair cross-complementing rodent repair deficiency, complementation group 8	Involved in preferential repair of active genes; repair of transcribed strand; interacts with CSB and TFIIH	2	S150C	167037

¹ Gene function from www.ncbi.nlm.nih.gov.

² Targeted nsSNPs with either variant-allele frequency ≥ 0.05 or predicted to have functional implications by SIFT or PolyPhen program.

³ None means that none reported or fit the selection criteria.

Table 3: NER nsSNPs in CaP risk and Functional Phenotype

Gene Symbol	rs#	Allele 1	Allele 2	Codon	Wild Type	Variant	Variant frequency	CaP risk (OR; 95% CI)	SIFT Score	PolyPhen Score	NERC Effect
<i>ERCC2</i>	1799793	G	A	312	<i>Asp [D]</i>	<i>Asn [N]</i>	0.41	0.78 (0.50-1.21) ¹	0.57 Tolerant	0.099 Benign	Yes ^{4,5}
<i>ERCC2</i>	1052559	A	C	751	<i>Lys [K]</i>	<i>Gln [Q]</i>	0.35	0.94 (0.63-1.40) ¹	0.45 Tolerant	0.181 Benign	Yes ^{4,5}
<i>ERCC4</i>	1800067	G	A	415	<i>Arg [R]</i>	<i>Gln [Q]</i>	0.09	1.37 (0.95-1.97) ²	0.03 Intolerant	1.357 Benign	No ⁵
<i>ERCC5</i>	17655	G	C	1104	<i>Asp [D]</i>	<i>His [H]</i>	0.46	0.84 (0.48-1.46) ¹	1.00 Tolerant	1.519 Possibly Damaging	Yes ⁵
<i>RAD23B</i>	1805329	C	T	249	<i>Ala [A]</i>	<i>Val [V]</i>	0.29	1.06 (0.79-1.42) ³	0.16 Borderline	1.290 Benign	No ⁵
<i>XPC</i>	2228000	C	T	499	<i>Ala [A]</i>	<i>Val [V]</i>	0.24	0.85 (0.48-1.51) ¹	0.04 Intolerant	1.097 Benign	Yes ⁵
<i>XPC</i>	2228001	A	C	939	<i>Lys [K]</i>	<i>Gln [Q]</i>	0.38	0.99 (0.68-1.44) ¹	0.00 Intolerant	0.900 Benign	No ⁵

¹ OR, odds ratio, recessive model, adjusted for age, benign prostatic hyperplasia, smoking history, and family history; CI, confidence interval.

² OR, *RQ* vs. *RR*, adjusted for age, benign prostatic hyperplasia, smoking history, and family history.

³ OR, dominant model, adjusted for age, benign prostatic hyperplasia, smoking history, and family history.

⁴ An effect on NERC was reported previously [51].

⁵ Based on our preliminary unpublished genotype data and NERC data from [59].