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THE ROLE OF AGING AND DNA REPAIR IN CHRONIC DISEASE

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ABSTRACT

We carried out a molecular epidemiological study of the DNA repair of photochemical damage as a risk factor in basal cell carcinoma (BCC). In that clinic-based case control study of 88 cases and 135 cancer-free control it was found that DNA repair in the controls declined linearly at a rate of 0.61% per year over a 30-60 year age group. However, repair in younger BCC cases, significantly less than their age-matched controls, did not decline at the same rate so that the repair differences between the cases and the controls disappeared as the cases grew older. Besides this age effect, the odds are high (5:1) that an individual with low repair overexposed to sunlight will have basal cell carcinoma. That these odds increase to 10:1 for females compared to male subjects led to the observation that repair may be sensitive to hormonal control. Because of the ease of BCC diagnosis it is possible to demonstrate significantly that the level of DNA repair directly influences the multiplicity of tumors. Further, both those cases and controls with a family history of BCC invariably have reduced levels of DNA repair ($p < 0.05$).

REPORT:

A method for measuring DNA excision repair in response to ultraviolet irradiation and chemical-induced DNA damage has been developed, validated, and field-tested in cultured human lymphocytes. The methodology is amenable to general population-based screening and can be used for these epidemiologic studies seeking to investigate associations between DNA repair proficiency and chronic disease susceptibility.

For the assay a damaged non-replicating recombinant plasmid DNA harboring a *chloramphenicol acetyltransferase (cat)* reporter gene is introduced into T-lymphocytes using DEAE-dextran/DNA complex short-term transfection conditions. Repair of the damaged bacterial *cat* gene is monitored proportionately as a function of reactivated CAT enzyme activity following a 40 h repair-expression incubation period. The response on the part of the cell is that of *host cell reactivation (HCR)* at the molecular level. The survival curves (including D_0) for the damaged cell, for HCR of damaged viruses and for this molecular assay are the same. Approximately fifty percent of the saturating input acetylCoA is found as the mono- and diacetylated chloramphenicol.

T-lymphocytes were chosen as surrogate cells for skin cancer for a number of reasons. Humans exposed to sunlight have demonstrable levels of UV photoproducts in their lymphocyte DNA. These cells are in equilibrium with virtually all cells in the body, they can pass through the blood-brain barrier and finally the DNA repair curves of circulating lymphocytes are the same as published repair data for established fibroblasts, lymphoblasts and fresh lymphocytes from patients with DNA repair deficiencies.

The validity of the approach was indicated by the assay's ability to discriminate XP virus-transformed lymphocyte cell lines of both severe (complementation groups A and D and moderate (complementation group C) excision repair deficiencies from repair-proficient cell lines. Identical results were observed when a mitogen-stimulated peripheral blood lymphocyte (PBL) culture from an XP-A (homozygous) patient was assayed concurrently with mitogen stimulated PBLs obtained from healthy individuals. The sensitivity of the assay was demonstrated with PBLs from obligate heterozygotes (enclosed reprint). The results of these studies identified at risk for basal cell carcinoma those young females over exposed to sunlight with low DNA repair capacity (Table 1) These and other data from the same publication exemplify the consistent precision of this assay in

which the variation amongst the controls is less than 5% generating confidence in the comparisons between cases and controls. It can also be seen from these data that the number of subjects is extremely small. There were only 3 subjects in the control category even though the comparisons between the two groups are *biochemically significant*. The differences between cases and controls are not *statistically significant* although they are *statistically valid*. Standard deviation is consistently $\pm 2\%$, hence, no error bars are necessary. Corroboration of the pilot studies, when applied to sample size calculations for stratification of the data according to gender, family BCC history, sunlight exposure (UV dosimetry), age, hormonal, dietary and drug histories required 150 cases of basal cell carcinoma and 250 of their case matched controls, was then sought.

Because of the design of the patient record data base in the JHU Dermatopathology Laboratory we had to individually examine 10,000 patient's records. Two thousand patients, were selected, their physicians contacted for permission to approach them through correspondence. Of these, 450 patients volunteered for the study. A clinic was made available for 4 months for visitation of the subjects after their work hours and consisted of a receptionist who guided the subjects through a detailed self-administered questionnaire. They were next examined by Dr. Evan Farmer, Professor of Dermatology, with the assistance of a nurse after which 30 cc of blood was drawn by a phlebotomist, coded and immediately fractionated by Ficoll gradients and the leucocyte fraction frozen to -80°C at the rate of 1°C per minute. We collected all the samples over a 10-week period.

The DNA repair assay used in the pilot study was then applied to 10 cc of blood equivalents of the T-lymphocytes obtained after PHAH-P stimulation. The remaining 20 cc (2×10^7 T-lymphocytes) of blood equivalents are maintained in the Johns Hopkins "Core Tissue Facility" by our technician who is in charge of blood collections, fractionations and record keeping of the "DNA repair Library" including these subjects and those of two other population studies (lung and colorectal cancer) under way. These cells will be available for this study as well for other interested scientists.

Age and sex adjusted odds ratios for interaction between DNA repair capacity and risk factors of BCC were estimated by logistic regression. Surprisingly there was an age related decline in this DNA repair capacity, amounting to close to 1.0 percent per year in the controls from 20-60 years of age (Figure 1). Reduced DNA repair was a particularly important risk factor for young BCC cases. Young BCC cases repaired DNA damage poorly when compared to controls. As the BCC patients aged, however, the differences between the cases and controls gradually disappeared. The normal decline in DNA repair with increasing age may account for the increasing risk of skin cancer which begins in middle age. These findings suggest that, in terms of DNA repair, the occurrence of skin cancer in the young, may represent premature aging. This reflects the appearance the young XP patients who develop tumors 30-40 years earlier than in the general population. Insights into the rate limiting steps in DNA repair progression between the two different biological states of aging and basal cell carcinoma is, therefore, of great interest.

The DNA repair capacity data differences between the pilot and population studies, obtained by different investigators using different control subjects of the same age and gender, were consistent and within 5-10% of each other. When multiple intra-assay tests were carried out on the same samples rankings were constant. Those who had the lowest, highest and average DNA repair maintained their rank order over four trials further emphasizing the value of this assay for epidemiological studies.

The age related effects on DNA repair are reflected in the multiple linear regression analyses in Table 2 in which the *p*-values are less than 0.05. Importantly, a family history for BCC appears to be a harbinger of reduced repair in both cases and controls (Table 2). This is reflected in

multiple linear regression analyses of DNA repair which suggests a sort of "molecular syllogism" for the data in Figure 2a. Here, the subjects with reduced DNA repair capabilities and overexposure to sunlight had an estimated risk of basal cell carcinoma more than 5-fold greater than the control group. Importantly, however, a risk was even greater (10-fold) in female subjects (Figure 2b). Parenthetically, the level of reduced DNA repair capacity significantly affects the increased number of multiple skin tumors ($p < 0.05$) after adjustment for age (*Cancer Research*, in press, preprint enclosed).

table 1

Plasmid HCR repair response (D_0 and %CAT₈₀₀) in male and female basal cell carcinoma cases and controls stratified by sunbathing frequency

	Case				Control			
	No.	%*	D_0 Mean $\pm$ SD	%CAT ₈₀₀	No.	%*	D_0 Mean $\pm$ SD	%CAT ₈₀₀
Male								
Rarely/occasionally	10	0.36	338 $\pm$ 60	9.9	7	0.25	332 $\pm$ 81	9.3
Frequently	4	0.14	361 $\pm$ 110	10.6	7	0.25	332 $\pm$ 43	9.9
Female								
Rarely/occasionally	15	0.41	308 $\pm$ 76	8.1	10	0.27	323 $\pm$ 69	8.8
Frequently	9	0.24	270 $\pm$ 55	5.8	3	0.08	341 $\pm$ 70	10.1

FIGURE 1

The gender orientation observed in this population study is consistent with the increased vulnerability of females observed in the pilot study. The questionnaire was modified, as a consequence of that validation study, to include those questions that deal with stages of oestrous, stages of menopause, use of oral contraceptives and estrogen supplementation. The data in Table 3 suggests that DNA repair is sensitive to hormones - an important clue as to loci for further laboratory investigations dealing with DNA repair regulation. Regulation is largely an unknown area of DNA repair in vertebrates and only partially understood in procaryotes and smaller eukaryotes. The data in Figure 3 supports the notion of the influence of estrogen in post-menopausal women in the age stratified repair data derived from Figure 1. The consistently lower repair observed in the female controls and the pre- and postmenopausal levels of repair serve to explain the crossover between the cases and controls at 55-years of age described in Figure 1.

The primary lymphocyte system is amenable to studying repair under non-growing conditions in cell culture. Lymphocytes have estrogen receptors, whereas, androgen receptors have been identified in primary skin fibroblasts. The role, therefore, of estrogens and androgens in the intracellular levels of DNA repair will be studied in primary and transformed lymphocytes and fibroblasts to measure responsiveness at the cellular level under a variety of biological situations. It

is also planned to determine whether hormones exert their effects at the level of receptor-independent adduction to DNA and/or through signal transduction requiring specific receptors.

If DNA repair is a primary target for apoptosis it can be anticipated that there should be a corresponding increase in damage persistence as a function of aging. These findings are supported by the observations of Cole *et al* at the Mutagenesis Unit, MRC Sussex, England) of a concomitant increase in mutation fixation of a number of genes at the rate corresponding to an increase of 1-2% per annum over a wide aging range.

table

2 Multiple linear-regression modeling of DNA repair related to risk factors among BCC and controls

Model*	Parameter	Estimate†	t value‡	P value§
1	Intercept	11.725	10.23	0.000
	Age in yr	-0.071	-3.47	0.000
	Sex	-0.409	-1.11	0.271
2	Intercept	10.445	6.96	0.000
	Age in yr	-0.047	-1.85	0.067
	Sex	-0.547	-1.26	0.211
3	Intercept	11.374	7.77	0.000
	Age in yr	-0.134	-3.09	0.003
	Sex	-0.360	0.84	0.404
4	Intercept	11.569	12.81	0.000
	Age in yr	-0.067	-4.24	0.000
	Sex	-0.423	-1.51	0.133
	BCC FH	-0.645	-1.93	0.055

All models but model 2 significantly differ from a model with no risk factors according to the *F* test. The variables included in these linear-regression models are as follows: dependent variable is percentage of CAT activity at a UV dose of 700 J/m² (continuous variable); independent variables are age (at assay, in yr); age of onset (age at first BCC, in group): 1, <35; 2, 35-44; 3, 45-54; 4, 55-60; sex: 1, male; 2, female; BCC family history (FH): 1, without family history of BCC, 2, with BCC family history.

*Model: 1, control only (*n* = 135) with *F* value = 6.35 and *R*² = 0.088 and *P* = 0.002; 2, BCC case only (*n* = 88) with *F* value = 2.17 and *R*² = 0.049 and *P* = 0.120; 3, BCC case only (*n* = 88) with *F* = 3.51 and *R*² = 0.111 and *P* = 0.019; 4, all subjects (*n* = 223) with *F* = 7.44, *R*² = 0.092 and *P* = 0.0001.

†Least-square estimate of regression coefficient from multiple linear-regression models.

‡Student's *t* test for the null hypothesis that the estimate is equal to zero.

§Two-sided Student's *t* test.

FIG. 2. Effect of DRC on risk of BCC: Relationship to number of severe sunburns in a lifetime. The reference group has an odds ratio of 1. The significantly increased age- and sex-adjusted odds ratio [(5.32; 95% confidence interval (CI), 2.04-13.9)] is seen only in those who had both low DNA repair and six or more severe sunburns in their lifetime. **, *P* < 0.01.

The persistence of UV-induced photoproducts, attributed to reduced DNA repair was also studied at the level of p53 genes in BCC. We analyzed 36 BCCs for p53 mutations, and a subset of these tumors for loss of chromosomes 17p and 9q. Sixty-nine percent of the BCCs had lost a 9q allele, with the common area of loss surrounding the putative gene for nevroid basal cell carcinoma, or Gorlin's Syndrome. Forty-four percent (16/36) of BCCs had a mutated 53 allele, usually opposite pyrimidine tracts, which is consistent with UV-induced pyrimidine dimer and 6-4 adduct generated mutations. Surprisingly, only one tumor lost a 17p allele, and in all BCCs only one p53 allele was inactivated. This is in direct contrast to other epithelial tumors, which usually progress by the inactivation of both p53 alleles. It is possible that the absence of clinical progression of BCC may be related to the lack of complete p53 inactivation in these tumors (*Cancer Research* in press, preprint enclosed).

Table 3
Effect of exogenous hormone on DNA repair in women (n=108)

Study Variable	Yes		No		Percent difference ^b	P-value ^c
	(n)	Mean $\pm$ SD ^a	(n)	Mean $\pm$ SD		
Premenopausal use of oral contraceptive	(45)	7.9 $\pm$ 2.1	(21)	7.5 $\pm$ 2.4	+ 5.3	0.567
Postmenopausal estrogen supplement	(28)	7.5 $\pm$ 2.1	(14)	6.0 $\pm$ 2.0	+25.8	0.038

^aMean of DNA repair response (percent CAT activity at UV dose to plasmid 700 J/m²) and its standard deviation. Means were chosen as estimates because the residual repair response follows an approximately normal distribution.

^bPercent difference between the means of cases and controls.

^cProbability derived from two-sided *t*-tests.

figure 3

Age-Related DNA Repair Decline by Sex
in BCC cases (87) and controls (137)

BCC = Basal Cell Carcinoma

Comparison of estrogen supplemental use in BCC cases and controls after diagnosed as having postmenopausal status, by age

	41-50		51-60	
	BCC case no. (%)	Control no. (%)	BCC case no. (%)	Control no. (%)
Total number	4 (100)	29 (100)	11 (100)	22 (100)
postmenopausal	2 (50)	7 (24)	11 (100)	16 (73)
estrogen use	1 (13)	7 (24)	9 (82)	10 (46)

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