DNA Repair Related to Multiple Skin Cancers and Drug Use

Qingyi Wei, Genevieve M. Matanoski, Evan R. Farmer, Mohammad A. Hedayati, and Lawrence Grossman

Departments of Epidemiology [Q. W., G. M. M.] and Biochemistry [Q. W., M. A. H., L. G.], School of Hygiene and Public Health, and Department of Dermatology, School of Medicine [E. R. F.], The Johns Hopkins University, Baltimore, Maryland 21205

ABSTRACT

Defective repair of sunlight-induced DNA photodamage, coupled with an unusually high occurrence of multiple primary basal cell carcinomas (BCCs), is the major characteristic of xeroderma pigmentosum. Our recent work has indicated that this etiological paradigm may apply to skin cancer patients without an apparent hereditary disease. The present study reports on an investigation of whether medications such as photosensitizing drugs (antibiotics, corticosteroids, and aspirin) modulate skin cancer risk through alterations in DNA repair capacity (DRC). Using a new DNA repair (host cell reactivation) assay with peripheral T-lymphocytes, we tested DRCs of 88 Caucasian BCC patients and 135 cancer-free controls. Subjects were between 20 and 60 years of age and free of known hereditary skin diseases. The age-adjusted means of DRC were calculated to compare repair levels associated with the use of specific drugs and hormones. Multiple linear regression models were used to correlate DRC with the number of skin cancers. The estimated odds ratio was used to describe the risk of BCCs. The distribution of DRCs of subjects was approximately normal, with a 5-fold variation between individuals. DRCs below the upper 30th percentile of controls were associated with an estimated 2.3-fold (95% confidence interval, 1.17-4.54-fold) increased risk for the occurrence of BCCs. The lower the DRC was, the greater the number of skin tumors in individuals (P < 0.05), after adjustment for age. Although supplemental vitamin use was associated with reduced risk of skin cancer, it was not associated with differences in subjects' DRCs. However, individuals who reported taking either tetracycline or estrogen, two photosensitizing drugs, had higher DRCs, compared with those who had not used these drugs. Low DRC or a family history of skin cancer increased the probability that patients who were overexposed to sunlight would have multiple BCCs. DNA repair levels may be influenced by the use of selected photosensitizing drugs and estrogen.

INTRODUCTION

Sunlight exposure was implicated in skin cancer risk as long ago as 1896 (1), but the link between deficient DNA repair and sunlight-induced skin cancer was not established until 1968, with the identification of a rare autosomal recessive syndrome, XP (2). Sunlight exposure coupled with a reduced ability to repair photochemically damaged DNA results in a about a 2000-fold greater risk of developing skin cancer in patients with XP, compared to the general population (3). The course of the disease is characterized by multiple primary skin tumors, especially BCCs (3). Recent studies indicate that, in addition to sunlight exposure, a family history of skin cancer (4-6) and reduced DNA repair (7-9) are risk factors for skin cancers in the general population as well. The fact that DNA repair levels vary in patients with XP (3) suggests that such variation may also occur in both patients with cancer and normal individuals, but few studies have investigated whether DNA repair plays a role in the etiology of skin cancer in non-XP patients. Our recent population-based study (6) suggested that low DRC may play a role in skin carcinogenesis in the general population as it does in XP patients.

Previous studies have linked multiple BCCs to family history (10) and HLA-DR (11, 12) in young individuals (13). These findings suggest that multiple skin cancers may be associated with some familial factors. If patients with nonhereditary skin cancer follow the paradigm of patients with XP, then the number of BCCs per individual should be associated with reduced DNA repair capacity. The current report examines the relationship between the number of BCCs and DNA repair in non-XP patients. In addition, other factors, such as the use of photosensitizing drugs, that may influence DNA repair or the risk of BCC are also examined.

MATERIALS AND METHODS

Study Subjects. The case-control study design was described previously (6). Briefly, 88 Caucasian patients (55% men), of age 20-60 years (mean, 48 years), with histopathologically confirmed primary BCCs were selected as the cases. They were relatively young in terms of mean age of onset, compared to a mean of 60 years for nonmelanoma skin cancer in the United States population (3). The 135 controls (mean age, 46 years) consisted of Caucasian patients from the same dermatology practices as the cases. The controls with pathological diagnoses of noncancer skin disorders, as described previously (6), were frequency matched to the cases by age (±5 years) and sex (50% men).

Clinical Visit. The procedures for clinic visits were described previously (6). Briefly, the procedures and protocols in this study were approved by the Johns Hopkins Joint Committee on Clinical Investigations. Cases and controls attended a special dermatology clinic for this study, where an informed consent was obtained from each subject. Subjects completed a structured self-administered questionnaire which contained questions regarding detailed medical history, including information on occurrence of any malignant tumors and use of medications such as prescription drugs, photosensitizing agents, vitamin supplements, oral contraceptives, and postmenopausal estrogen supplements. Information about the time and frequency of daily use and current use was obtained. A family history of cancer and other chronic diseases was also recorded for each individual. Skin examination by study dermatologists included a classification of natural hair color, eye color, and cutaneous sun sensitivity (Fitzpatrick's clinical skin typing) (14) and an identification and diagnosis of all sunlight-related skin lesions, as described previously (6). The attending dermatologist verified the signs for reported history of skin malignancies, including BCC, squamous cell carcinoma, and melanoma, for each subject. The subjects were examined for signs of surgical removal of previous skin tumors and the presence of any current skin cancers. Those controls with lesions which were suggestive of skin cancers but could not be confirmed through dermatopathology reports were excluded. To the extent possible, all clinical examinations were conducted without knowledge of the subjects' pre-existing pathology reports.

DNA Repair Assay. A 30-ml sample of peripheral blood was drawn from each subject for the DNA repair assay. The lymphocytes were isolated and frozen according to the procedures described previously (6, 7). Briefly, the host cell reactivation assay measures the ability of host peripheral blood lymphocytes to repair a UV-damaged reporter gene inserted into a vector plasmid which is transfected into the host T-lymphocytes. The reporter gene codes for the bacterial enzyme CAT. The ability of lymphocytes to repair UV-induced DNA damage is expressed as the percentage of the reactivated CAT activity of damaged relative to undamaged (baseline) genes during a 40-h period of gene repair and expression. For instance, the overall mean of CAT activity at a UV dose of 700 J/m² for 135 controls is 7.8%, relative to that at a zero dose (100%) for the same individuals. The measurement is independent of cell culture.

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conditions and immunological function (6). (The protocols for the CAT assay are available from L. G. on request.)

Statistical Analysis. Univariate analyses were used to explore the distribution of various kinds of drug use and DNA repair measurements. A series of contingency tables for selected variables including DNA repair were constructed, and χ² tests were used to examine the significance of any associations. The adjusted odds ratio and its 95% confidence interval were calculated using a logistic regression model (15). A correlation analysis was used to identify any correlation among related variables. Because of an age-related decline in DNA repair in this population (6), co-variance analysis (16) was used to calculate the age-adjusted least-squares mean of the DNA repair levels. The association between the level of DNA repair and the number of primary BCCs was evaluated by fitting a linear regression model treating the number of tumors as a continuous variable and controlling for age. The age variable was the age at the time when the blood sample was drawn, unless otherwise stated. The age-adjusted mean levels of DNA repair were compared with the use of various drugs. All tabulations and statistical analyses were performed with the SAS statistical package (16).

RESULTS

Basic Characteristics. Cases and controls were similar in age, sex, area of residence, education, household income, smoking, and alcohol consumption, as described previously (6). The percentages of cases and controls who had ever had an outdoor job for at least 1 year were about the same (24%). Cases were statistically more likely than controls to report characteristics known to predispose subjects to the risk of skin cancer, such as blue eyes, childhood freckling, and low tanning ability with more lifetime sunburns (data not shown). In addition, cases were significantly more likely to report a family history of skin cancer (33%) than controls (16%). These findings confirm that known risk factors for skin cancer were present in this population.

Low DNA Repair Capacity as a Risk Factor. In general, individual DRC values varied in this population. At a UV dose of 700 J/m², the residual DRCs for both cases and controls, relative to a zero dose, were in the range of 3–15%, which may be subject to the age effect (6) (Fig. 1). Compared to controls, the DRC of cases was shifted to the low end of the range. The cases had a mean DRC (7.4%) lower than that of the controls (7.8%). This difference was only marginally significant (P = 0.097). In previous reports (6), we used 50% of control DRC levels as a cutoff value to facilitate the evaluation of modification effects on DRC. In this analysis, we tried to find a cutoff value of DRC to maximize the risk for BCC. We found that individuals who had a DNA repair level below the upper 30th percentile of the controls had a >2-fold increased risk for BCC (odds ratio, 2.3; 95% confidence interval, 1.2–4.5; adjusted for age) (Table 1).

The number of tumors was counted according to the number of positive sites recorded in the pathology reports of skin biopsies from each individual at different calendar times. Thirty-nine % of the cases had multiple tumors and 80% of the multiple tumors were diagnosed at different calendar times. Among cases with multiple tumors, 45% reported previously treated BCCs, which were consistent with the signs of surgical removal. Compared to controls, those cases with increased numbers of tumors had decreased DRC (Table 2). The cases with four or more tumors had significantly lower mean levels of DNA repair, compared to that of the controls (P < 0.05). The trend was tested by linear regression analysis, which showed that the DNA repair capacity, independently of age, significantly decreased as the number of tumors increased (P < 0.05).

Family History of Skin Cancer as a Risk Factor. We previously reported that in this population more case subjects younger than 45 years of age reported a family history of skin cancer than did older cases and controls (6). In the current analysis (Table 2), the proportion of case subjects who reported a family history of skin cancer increased as the number of BCCs increased. This association was statistically significant (P < 0.01) and the groups were similar in age. This finding is consistent with the previous finding that a family history of skin cancer is an independent predictor of an individual’s DNA repair capacity (6).

Effect of Vitamin Supplements. A vitamin user was defined as an individual who regularly used supplemental vitamins during the 5 years prior to the clinical visit. About 39% of the controls and 28% of the cases used multiple vitamins regularly. The most frequently used vitamins were A, C, E, and multivitamin. The use of various vitamins was highly intercorrelated. For example, 75% of the vitamin E users also used vitamin C (r = 0.34, P < 0.001). We have observed an association between vitamin supplements and risk for BCC in this

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**Fig. 1.** Percentage distribution of host cellular DNA repair capacities (CAT activity) at a UV dose of 700 J/m² among BCC patients and their controls.
The logistic regression model. Being DRC and the independent variable being number of BCCs, controlling for age and population. However, in the current analysis the users and nonusers than did controls, regardless of vitamin supplementation (data not shown). Among the controls showed no difference in the mean level of DRC; cases in general had statistically nonsignificantly lower DRC values least 1 month in the last 5 years, as described previously (6). Tetracycline and corticosteroids (cortisone and hydrocortisone) were the most frequently used photosensitizing drugs reported by the subjects. Neither of these photosensitizing drugs appeared to be associated with increases did not reach a level of statistical significance.

**Effect of Photosensitizing Drugs.** Similar proportions of controls (34%) and cases (33%) reported the use of regular medications for at least 1 month in the last 5 years, as described previously (6). Tetracycline and corticosteroids (cortisone and hydrocortisone) were the most frequently used photosensitizing drugs reported by the subjects. Neither of these photosensitizing drugs appeared to be associated with an increased risk of skin cancer. Almost equal proportions (11%) of cases and controls used tetracycline. The mean DRC of nonusers for both cases (by 11%) and controls (by 16%), although the increases did not reach a level of statistical significance.

**Effect of Estrogen Replacement in Postmenopausal Women.** Female subjects were defined as postmenopausal if they had been identified as menopausal by their physicians before the clinical visit. The use of oral contraceptives and estrogen replacement therapy was determined by questionnaire. Forty-five % of the female cases were postmenopausal, compared to 34% for the controls. Among premenopausal women, there were almost equal percentages (68%) of cases and controls who reported ever using oral contraceptives. Among postmenopausal women, there was a slight difference in the proportion of cases (61%) and controls (74%) who reported using estrogen replacement, but there was no difference in the average age (about 47 years) at initiation of estrogen replacement therapy in the two groups. The average current age was 54.7 years for cases and 52.8 for controls. When all subjects were stratified according to menopausal status, there was a 25% relative increase in the mean level of DRC ($P < 0.05$) for estrogen users, compared with nonusers, among postmenopausal women. In contrast, only a 5.3% relative increase in repair was observed in oral contraceptive users, compared to nonusers, among premenopausal women (data not shown). The increased levels of DRC for estrogen users were consistently observed in both cases and controls. It appears that the increase in DRC may be contributed by older women whose age-related decrease in DRC was compensated for by estrogen use. After adjustment for age, however, the previously estimated 25% relative increase was reduced to 21% ($P = 0.055$), indicating that the estrogen effect on DRC was not substantially confounded by age (Table 4).

**DISCUSSION**

This study was designed to investigate whether low DNA repair was associated with increased risk of multiple skin tumors in non-XP patients. As demonstrated in our previous report (6), skin cancer in the general population appears to be due not only to sunlight exposure but also to reduced levels of DNA repair, as seen in XP patients. In the present study, we demonstrated that DRCs varied among individuals and low DRC was associated with an increase in the number of BCCs.

**Effect of Estrogen Replacement in Premenopausal Women.** There were 3 women whose age-related decrease in DRC was compensated for by estrogen use. After adjustment for age, however, the previously estimated 25% relative increase was reduced to 21% ($P = 0.055$), indicating that the estrogen effect on DRC was not substantially confounded by age (Table 4).

**Table 3** Effect of drug use on host DNA repair capacity (CAT activity)

<table>
<thead>
<tr>
<th>User</th>
<th>n</th>
<th>CAT activity (%)</th>
<th>Nonuser</th>
<th>n</th>
<th>CAT activity (%)</th>
<th>Difference (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>Control</td>
<td>8</td>
<td>8.4 ± 2.2</td>
<td>66</td>
<td>7.7 ± 2.1</td>
<td>+9.0</td>
<td>0.424</td>
</tr>
<tr>
<td>Case</td>
<td>6</td>
<td>9.4 ± 1.9</td>
<td>49</td>
<td>7.0 ± 1.8</td>
<td>+34.3</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>14</td>
<td>8.7 ± 2.1</td>
<td>115</td>
<td>7.4 ± 2.1</td>
<td>+17.6</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Control</td>
<td>6</td>
<td>8.1 ± 2.6</td>
<td>66</td>
<td>7.7 ± 2.1</td>
<td>+5.2</td>
<td>0.630</td>
</tr>
<tr>
<td>Case</td>
<td>3</td>
<td>7.0 ± 1.8</td>
<td>49</td>
<td>7.0 ± 1.8</td>
<td>+0.0</td>
<td>0.959</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>9</td>
<td>7.6 ± 2.0</td>
<td>115</td>
<td>7.4 ± 2.1</td>
<td>+2.7</td>
<td>0.762</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>Control</td>
<td>9</td>
<td>8.8 ± 2.6</td>
<td>66</td>
<td>7.7 ± 2.1</td>
<td>+15.8</td>
<td>0.112</td>
</tr>
<tr>
<td>Case</td>
<td>10</td>
<td>7.8 ± 1.8</td>
<td>49</td>
<td>7.0 ± 1.8</td>
<td>+11.4</td>
<td>0.261</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>19</td>
<td>8.2 ± 2.0</td>
<td>115</td>
<td>7.4 ± 2.1</td>
<td>+10.8</td>
<td>0.118</td>
<td></td>
</tr>
</tbody>
</table>

Users were those who used any drug for at least 1 month in the last 5 years. Relative difference was calculated as (user mean - nonuser mean)/nonuser mean × 100%. Probability derived from two-sided $t$ tests for means of user versus nonuser.

**Table 4** Effect of oral contraceptive (OC) and estrogen use on host DNA repair capacity (CAT activity)

<table>
<thead>
<tr>
<th>User</th>
<th>n</th>
<th>CAT activity (%)</th>
<th>Nonuser</th>
<th>n</th>
<th>CAT activity (%)</th>
<th>Difference (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal OC use</td>
<td>Control</td>
<td>30</td>
<td>7.8 ± 2.0</td>
<td>14</td>
<td>7.9 ± 2.2</td>
<td>-1.3</td>
<td>0.865</td>
</tr>
<tr>
<td>Case</td>
<td>15</td>
<td>7.7 ± 1.9</td>
<td>7</td>
<td>7.4 ± 1.9</td>
<td>+4.1</td>
<td>0.742</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>45</td>
<td>7.8 ± 2.1</td>
<td>21</td>
<td>7.7 ± 2.1</td>
<td>+0.6</td>
<td>0.945</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal estrogen use</td>
<td>Control</td>
<td>17</td>
<td>7.7 ± 2.0</td>
<td>6</td>
<td>6.3 ± 2.0</td>
<td>+22.2</td>
<td>0.173</td>
</tr>
<tr>
<td>Case</td>
<td>11</td>
<td>6.9 ± 2.2</td>
<td>7</td>
<td>6.1 ± 2.2</td>
<td>+13.1</td>
<td>0.501</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>28</td>
<td>7.4 ± 2.1</td>
<td>13</td>
<td>6.1 ± 2.1</td>
<td>+23.3</td>
<td>0.015</td>
<td></td>
</tr>
</tbody>
</table>

Relative difference was calculated as (user mean - nonuser mean)/nonuser mean × 100%. Probability derived from two-sided $t$ tests for means of user versus nonuser.

**References**

1. Unpublished observations.
2. Family history was collected as nonmelanoma skin cancer in first- or second-degree relatives.
3. Residual DNA repair capacity at a UV dose of 700 J/m².
4. Mean ± SD.
5. Student’s $t$ test for the mean DRC, comparing each case group to the control.
6. $X^2$ test (degrees of freedom = 4) for the association between number of BCCs and family history.
7. Test for trend using a multiple linear regression model, with the dependent variable being DRC and the independent variable being number of BCCs, controlling for age and sex.
In addition, a positive family history of skin cancers also was associated with increased numbers of tumors, suggesting possible inherited factors responsible for the occurrence of multiple skin cancers. Munch-Peterson et al. (9) suggested that a reduced capacity to repair DNA might be related to an increased number of skin cancers. They reported that low repair capacities, as indicated by UV-induced DNA synthesis and lower UV tolerance, occurred only in patients \((n = 10)\) with both multiple BCCs and squamous cell carcinomas and, unlike this study, not in patients with only multiple BCCs \((n = 19)\). The limited statistical power due to the small sample size made it be associated with the number of tumors. Our findings in the current study provide evidence that DRCs are related to familial factors, as indicated by familial skin cancers, and that DNA repair is an important etiological factor in skin carcinogenesis. More studies of DNA repair in those individuals who have multiple skin cancers and positive HLA antigens are warranted.

Several epidemiological studies have indicated that vitamins A, C, and/or E may have a protective effect against certain cancers, but few provide evidence for such an effect in skin cancer patients. These anticancer effects are purported to be due to the inactivation of reactive molecules \((17)\), e.g., singlet oxygen or free radicals, and immunological enhancement \((18)\). The present study examined the possibility that vitamins might have an anticancer effect through alteration of DNA repair. We found that there was no apparent effect on DNA repair capacity associated with vitamin supplementation in the last 5 years prior to the onset of skin tumors. However, this conclusion must be regarded as tentative because total dietary vitamin intake was not assessed and serum levels of antioxidants were not evaluated in this study.

Regarding photosensitizing drugs such as tetracycline and estrogen, there are no reports linking these drugs directly to a increased risk of skin cancer, although they are known to increase sunlight sensitivity. Paradoxically, whereas tetracycline causes high skin sensitivity to sunlight, the sensitivity appears to be related to an increase in the ability to repair sunlight-induced damage to DNA. This may explain why no direct association has been found between this drug and risk of skin cancer. The increased DRC in the subjects who used tetracycline should be interpreted with caution due to the limited number of users and lack of information on the dose being taken. The mechanism of how tetracycline might increase DRC is also unknown. A large sample of users is needed so that adjustment for other potential confounding variables, e.g., timing of the drug use, could be completed.

The increase in DRC associated with postmenopausal estrogen replacement suggests that the level of DNA repair may be sensitive to hormonal control, but the mechanism is unknown. It appears that estrogen replacement offsets some age-related decline in DNA repair capacity among postmenopausal women. It has also been suggested that estrogen can change cellular function through its receptors \((19)\). This could lead to changes in cellular DNA repair activity as well, because alterations of intracellular locations of DNA repair enzymes and enzyme concentrations may influence DNA repair capacity \((20)\). Therefore, possible hormone-related mechanisms controlling the regulation of cellular DNA repair activity may be involved.

Previously we demonstrated that age is one of the major factors that influences host DNA repair capacity \((6)\). To the best of our knowledge, no studies using this host cell reactivation assay have investigated factors that may affect host DNA repair. Therefore, the findings in this report need further verification. Further research might address the following questions: Is DRC inducible by photosensitizing drugs such as tetracycline? Is it transiently stimulated or is it stimulated on a long term basis by hormones? Is altered photosensitivity from drug-taking related to increased or decreased risk of skin cancer? These questions will be relevant for the clinical use of such medications.

**ACKNOWLEDGMENTS**

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