DNA Repair Capacity Correlates with Mutagen Sensitivity in Lymphoblastoid Cell Lines\textsuperscript{1,2}

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Abstract

This study describes a correlation between cellular DNA repair capacity and the frequency of mutagen-induced in vitro chromosomal breaks in selected lymphoblastoid cell lines. Two assays, host cell reactivation (HCR) assay for measuring cellular DNA repair capacity and in vitro mutagen sensitivity assay, have recently been shown to be useful biomarkers for such susceptibility. Increased in vitro mutagen sensitivity, measured by the number of induced chromatid breaks, has been postulated to reflect decreased capacity of DNA repair, as measured by the HCR assay. However, these two assays have not been examined in parallel to test this hypothesis. In this study, we performed both assays in 16 established lymphoblastoid cell lines derived from patients with xeroderma pigmentosum (n = 3), ataxia telangiectasia (n = 2), head and neck cancer (n = 3), and melanoma (n = 2), and from normal human subjects (n = 6) using UV light, 4-nitroquinoline-1-oxide (4-NQO; an UV-mimetic agent), and \gamma-irradiation as the test agents. The measurements from the HCR assay correlated significantly with the frequency of chromatid breaks induced by either UV irradiation (r = -0.69; P < 0.01) or 4-NQO (r = -0.70; P < 0.01). Although published data suggest that damage induced by UV and 4-NQO may be repaired by different pathways, the two agents induced similar frequencies of chromatid breaks (r = 0.68; P < 0.01) in the tested cell lines. Our results also indicated that the HCR assay is not suitable to test agents that cause DNA strand breaks, such as \gamma-irradiation, whereas the mutagen sensitivity assay is. Although reduced cellular DNA repair capacity correlated with increased frequency of mutagen-induced chromatid breaks in these cell lines, these two assays have different sensitivities in measuring the repair of damage induced by different carcinogens; therefore, the use of both assays is recommended for future molecular epidemiological studies of cancer susceptibility.

Introduction

Most common cancers are considered to be the result of an interaction between environmental exposure and host susceptibility (1, 2). Inherited host susceptibility to carcinogens is well documented (3–6) and implicated in all stages of carcinogenesis (7).

Investigators working in the field of DNA repair and sensitivity to UV light are familiar with several phenomena: (a) patients with XP\textsuperscript{a} are extremely sensitive to UV; (b) XP patients are deficient in repair of DNA damage induced by UV (8); (c) XP patients are not sensitive to ionizing radiation, whereas patients with AT (9) exhibit a reverse property (i.e., highly sensitive to ionizing radiation but not to UV); and (d) the frequencies of skin malignancies increased when persons of Nordic origin emigrated to subtropical or tropical regions, apparently suffering from unaccustomed harsh UV exposure. These facts strongly suggest that DNA repair capability may vary among individuals and that such differences may well be genetically determined and, therefore, contribute to susceptibility to cancer induced by environmental agents such as UV. Assays that facilitate the identification of these susceptible individuals will enhance the effectiveness of cancer prevention strategies.

One of us (10) proposed a working hypothesis that differential sensitivity to mutagens among individuals may be determined by each individuals' DNA repair capability. However, such a hypothesis must be supported by experimental data. The bleomycin assay (11, 12) and the 4-NQO assay (13) were subsequently developed for testing this hypothesis in population studies. These assays use short-term lymphocyte cultures for assessing in vitro sensitivity [measured as the number of induced chromatid breaks] to the test mutagens (14, 15). It has been shown that the frequency of bleomycin-induced chromatid breaks is a useful biomarker of host susceptibility to cancer in case-control studies of lung and head and neck cancers (16, 17).

By the same token, another assay, the HCR assay, was developed by other investigators (18) to measure the extent of the overall DNA repair process and efficiency with a transient expression vector harboring a reporter gene, CAL. In this assay, the plasmid pCMVcat that has been damaged (or inactivated) by test carcinogens is transfected into the host cells that are not damaged by the carcinogens. The reactivation of the damaged CAL in these cell lines correlates with the efficiency of DNA repair. This assay was developed to be a useful screening tool for individuals predisposed to cancer, but it requires further study to determine its sensitivity and specificity.

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\textsuperscript{4} The abbreviations used are: XP, xeroderma pigmentosum; AT, ataxia telangiectasia; 4-NQO, 4-nitroquinoline-1-oxide; HCR, host cell reactivation; CAT, chloramphenicol acetyltransferase; b/c, chromatid breaks per cell.
plasmids allows measurement of the normal DNA repair capacity of the host cells. This assay was used in a molecular epidemiological study to evaluate individual variation in DNA repair capacity and considered as a potentially useful biomarker of genetic susceptibility to skin cancer (19).

Both the mutagen sensitivity assay and the HCR assay seemingly confirmed the idea that among human individuals there are variations in response to mutagens or in inherited DNA repair capacity and that such phenotypes might be associated with cancer risk of specific organs. In this study, we used the following assay methods: (a) 4-NQO-induced chromatid breakage rates; (b) UV light-induced chromatid breakage rates; and (c) the HCR assay, which was performed on 16 selected lymphoblastoid cell lines derived from XP, AT, and cancer patients and from normal subjects. XP and AT cells with known specific repair deficiency allow cross-references for evaluating specific repair activity and were used as the positive or negative controls in both the mutagen sensitivity assay and the HCR assay. If a degree of correlation exists between the test methods, then a higher correlation would be expected (i.e., the higher the mutagen sensitivity the lower the DNA repair capacity or vice versa).

Materials and Methods

Cell Lines and Cell Culture. Two types of cell lines were used in the experiments: lymphoblastoid cell lines obtained from Human Genetic Mutant Cell Repository (Camden, NJ) of the National Institute for General Medical Sciences and EBV-transformed lymphoblastoid cell lines established in our laboratory from cancer patients and healthy blood donors (12). A total of 16 lymphoblastoid cell lines were used: three XP lines [XP-A (GM02345a), XP-C (GM02246b), and XP-D (GM02485b)] deficient in nucleotide excision repair; two AT lines [AT-1 (GM01525c) and AT-2 (GM01526c)] with hyper-radiosensitivity; and 11 lymphoblastoid cell lines, which included three head and neck cancer patients (3548p, 3513p, and 3585p; see Ref. 12). The cell lines used in the experiments: lymphoblastoid cell lines obtained from Human Genetic Mutant Cell Repository (Camden, NJ) of the National Institute for General Medical Sciences and EBV-transformed lymphoblastoid cell lines established in our laboratories from cancer patients and healthy blood donors (12). A total of 16 lymphoblastoid cell lines were used: three XP lines [XP-A (GM02345a), XP-C (GM02246b), and XP-D (GM02485b)] deficient in nucleotide excision repair; two AT lines [AT-1 (GM01525c) and AT-2 (GM01526c)] with hyper-radiosensitivity; and 11 lymphoblastoid cell lines, which included three head and neck cancer patients (3548p, 3570p, and 3640p; see Ref. 12), two melanoma patients (4828p and 4895p; unpublished lines), and three commercial normal lines (GM0131a, GM03798, and GM00892b), and three normal donors (3402p, 3513p, and 3585p; see Ref. 12). The cell lines grown in suspension were cultured at 37°C in a 5% CO₂ atmosphere in RPMI 1640 supplemented with 15% fetal bovine serum (GIBCO, Grand Island, NY) without antibiotics.

Mutagen Sensitivity Assay. The laboratory protocols of this assay have been described in detail elsewhere (11, 13). Briefly, approximately 5 × 10⁶ cells were inoculated into T-25 plastic culture flasks containing 10 ml of medium. Within 24 h of incubation, vigorous cellular growth was obtained. The cells were then treated or irradiated with agents known to induce DNA damage in certain cells: 4-NQO (Sigma Chemical Co., St. Louis, MO), UV light (unfiltered germicidal UVC and UVB lamps, 15 W; Sankyo Denki Co. Ltd., Japan), and γ-irradiation from a cesium 137 source (Cesium Irradiator Mark 1, Model 30; J. L. Shepherd & Associates, Glendale, CA).

For 4-NQO treatment, the drug was dissolved in acetone at a concentration of 1 × 10⁻³ M, stored at −20°C, and warmed to 37°C before use. The stock 4-NQO solution was added to the cell culture at a final concentration of 2 × 10⁻⁶ M, and 24 h later the cells were harvested. For UV irradiation, 2 × 10⁶ cells in 2 ml of culture medium (instead of a salt solution, which causes extra loss of cells due to more washes needed for the culture after irradiation) were transferred to a 60-mm tissue culture dish and placed uncovered in a biological hood for 30; J. L. Shepherd & Associates, Glendale, CA).

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Cells were harvested according to the standard procedures (Refs. 12 and 13; i.e., 1 h of Colcemid arrest, hypotonic KCl pretreatment, Carnoy fixation and washing, and then air-dried). Five slides were prepared for each assay. The slides were stained with 4% Giemsa without banding, and a total of 50 well-spread metaphases from each sample were examined with a 100× bright-field objective. The procedure used to record chromatid breaks followed the criteria described elsewhere (12, 13). The number of chromatid breaks was averaged into the number of b/c, with ≥0.8 b/c considered sensitive (≥1.0 b/c, hypersensitive). Three independent assays were performed for each cell line unless otherwise stated, and the average of the three b/c values was used for statistical analysis.

HCR Assay. Cellular DNA repair capacity was measured by the HCR assay, which was performed as described elsewhere (18). Purified plasmids (pCMVcat; 5 kb) were dissolved in TE solution at a concentration of 50 μg/ml, and 2-ml aliquots of the solution were placed in 35-mm tissue culture dishes. For UV irradiation treatment, the dishes were uncovered and separately irradiated with 0, 100, 200, 400, or 800 J/m² of UV light at a wavelength of 254 nm. For γ-irradiation treatment, the dishes remained covered and were separately irradiated with 0, 62.5, 125, 250, or 500 Gy. These treatments were performed in one batch for all CAT assays. After treatment, the plasmids were checked with 0.8% agarose gel for conformational changes. The treated plasmids or untreated plasmids (as controls) were transfected into the cells (approximately 0.25 μg plasmids per 2 × 10⁶ cells for each determinant) by the diethylaminoethyl dextran procedure (20). The transfections were performed in triplicate for each dose.

After transfection, another 48-h incubation was allowed for the repair of damaged plasmids and CAT gene expression, and the activity of CAT enzyme in the cell extract was estimated by measuring the radioactivity from [¹⁴C]mono- and diacetyl-chloramphenicol formed after an enzymatic reaction catalyzed by the CAT enzyme. The average CAT activity for the triplicates was calculated, and the ratio of CAT activity of treated plasmids to that of untreated plasmids was calculated as the percentage of residual repair activity (percentage of CAT activity) for the estimation of DNA repair capacity at a given dose. The experiments were repeated three times, and the averages were used for statistical comparisons.

Statistical Analysis. Correlation analyses were performed for DNA repair capacity and mutagen sensitivity estimates, and correlation coefficients were calculated. Student’s t test and Wilcoxon’s signed ranking test were performed to evaluate the differences among the estimates obtained from the two assays.

Results As expected in the mutagen sensitivity assay, AT cell lines, deficient in the repair of DNA damage induced by ionizing radiation, were not sensitive (b/c < 0.8) to UV irradiation up to a UV dose of 15 J/m², whereas XP cell lines, deficient in nucleotide excision repair, were hypersensitive (b/c ≥ 1.0) to UV irradiation at a dose of 5 J/m² (Fig. 1A). In these XP cells, a UVC dose of 10 J/m² induced cell arrest, leaving few met-
assays were then performed for each of the 16 cell lines using a UVB dose of 200 J/m² instead of 150 J/m² to increase the effect and a 4-NQO dose of $2 \times 10^{-6}$ M.

As shown in Fig. 2, the plasmids treated with γ-irradiation underwent dramatic conformation changes, which indicate increased percentages of the relaxed or linearized form, indicating strand breaks, with a dose-response pattern (0, 62.5, 125, 250, and 500 Gy; Fig. 2B). On the other hand, UVB dosages up to 800 J/m² did not produce conformational changes (Fig. 1B).

As a result of relaxation (Form II) or linearization (Form III) of plasmids induced by γ-irradiation, the HCR assays with plasmids treated with dosages up to 500 Gy did not differentiate DNA repair capacities between repair-deficient (AT) and repair-proficient (normal) cells (Fig. 3B) compared with the repair of UVC-induced damage (Fig. 3A). These results suggested that plasmids with single-strand or double-strand breaks were not transfectable, so that the expression of the CAT only reflected the plasmids of the supercoiled form (Form I) that were transfected. This may present limitations in the application of the HCR assay to measure the repair of radiomimetic damage.

For plasmids treated with UVC, XP cells had poor repair capacity, as expected, whereas AT cells had repair levels that were similar to those of apparently normal cells (Fig. 3A). These results are consistent with those from the mutagen sensitivity assays of cells treated with UV irradiation [Fig. 1; i.e., AT cells are sensitive to γ-irradiation (Fig. 1C) but not UVC irradiation (Fig. 1A and 1B)]. Taken together, these data provide evidence for the association of poor DNA repair capacity with increased mutagen sensitivity.

![Fig. 1. Dose-response curves of chromatid breaks induced by UVC (A), UVB (B), and γ-irradiation (C) in XP, AT, and apparently normal (GM) lymphoblastoid cell lines. AT cells were sensitive to γ-irradiation, whereas XP cells were sensitive to UV irradiation. Each point represents two independent assays.](image)

![Fig. 2. Effect of UV and gamma irradiation on plasmid pCMVcat. M, a molecular weight marker (lambda/HindIII). A. Lanes 1–5, UVC dosages of 0, 100, 200, 400, and 800 J/m². Although a UVC dose of 400 J/m² to pCMVcat corresponds to a loss of >95% of DNA repair capacity in XP cells compared to ~55% in other cells (Fig. 3A), there was no substantial change in the conformation of plasmids (form I, supercoil only) irradiated with up to 800 J/m² UV irradiation. B. Lanes 1–5, γ-irradiation dosages of 0, 62.5, 125, 250, and 500 Gy. In contrast with UV irradiation, γ-irradiation up to 250 Gy caused substantial relaxation (form II) and linearization (form III) of the plasmids, and no supercoil plasmid (form I) remained at 500 Gy because of single- and double-strand DNA breaks.](image)
The HCR assays with plasmids damaged by 800 J/m² of UVC irradiation were performed on all 16 cell lines and correlated with the results from the mutagen sensitivity assay of the same cells treated with UVB (200 J/m²) or 4-NQO (2 × 10⁻⁶ M). As shown in Fig. 4, the capacity to repair UV-induced damage to the plasmids is inversely correlated with the number of chromatid breaks induced by UV irradiation (r = -0.70; P < 0.01) and by 4-NQO (r = -0.69; P < 0.01). The Wilcoxon’s signed ranking tests were marginally significant for both of these two test mutagens (P < 0.06) in relationship to DNA repair capacity. In addition, the sensitivity to mutagens of cells treated with UV correlated also significantly with that of cells treated with 4-NQO (r = 0.68; P < 0.01), with a borderline significant ranking test (P < 0.07). These results suggest that the two assays are equally capable of differentiating repair-deficient cell lines but are not in good agreement in distinguishing cell lines with mild differences in DNA repair capacity from apparently normal cells. The differences in the ranking of measurements by the two assays may be due to the fact that the HCR assay measures the repair capacity for damage to exogenous DNA, whereas the mutagen sensitivity assay measures chromatid breaks as a result of both repair of damage to genomic DNA and response to cytotoxic effect of the test agents.

Additional t tests were performed for multiple comparisons between grouped cell lines. As shown in Table 1, XP cell lines were shown to have significantly lower DNA repair capacity or higher mutagen sensitivity (P < 0.005) than apparently normal cells by the two assays, respectively. In addition, melanoma lines were also identified as borderline sensitive to UV and head and neck lines to 4-NQO by the mutagen sensitivity assay but not by the HCR assay.

Discussion
Our results indicate that in DNA repair-deficient cells, the impaired ability to repair UV-induced damage is associated with increased frequency of chromatid breaks induced by UV and a UV-mimetic agent (4-NQO). These results provide evidence to support the hypothesis that increased in vitro mutagen sensitivity is the consequence of inadequate host cell repair ability. It is also consistent with the evidence that genetic alterations, both chromosome aberrations and gene mutations that may result from inadequate repair, are involved in carcinogenesis (21).

Advances in applied cytogenetics, such as the mutagen sensitivity assay, facilitate the detection of chromosomal aberrations in normal tissues of individuals who may be susceptible to cancer. Increased in vitro sensitivity to bleomycin has been shown to be associated with risk not only of primary (16, 17)
but also of second upper aerodigestive tract cancers (22). Although the frequency of chromosome aberrations or chromatid breaks measured by the mutagen sensitivity assay is a nonspecific marker for such susceptibility, the assay has the flexibility to accommodate the assessment of cancer risk related to exposures to different carcinogens. However, the limitation of the assay is that the aberrations or breaks can be monitored only in metaphase. In addition, many subtle modifications of DNA, such as interstrand or intrastrand cross-links (e.g., dimers) or point mutations, that contribute to carcinogenesis are not detected in this test system. Therefore, it is desirable to use the mutagen sensitivity assay in parallel with other assays that may be complementary, such as the HCR assay. This will provide more information about the molecular mechanisms underlying carcinogenesis.

The recent progress in research on DNA repair genes and their molecular mechanisms in maintaining genomic stability has pointed to a new avenue for their role in carcinogenesis (23). Although evidence for the association between DNA repair deficiency and carcinogenesis has been well studied in rare genetic disorders (24), information about this association in the general population is limited (19). The evaluation of DNA repair capacity in the general population depends on reliable assays that are simple and convenient. There are several ways of measuring cellular DNA repair: rate of damage removal (25), damage-dependent or repair-dependent formation of single-strand breaks that reduce plasmid transfer frequency (31), uptake of substrate for unscheduled DNA synthesis (27), gene expression level from HCR of the damaged reporter gene (18), and gene-specific or strand-specific repair (28). Each assay measures a unique rate-limiting step in the repair process. The HCR assay has been shown to be suitable for population studies in which time, cost, and the repeatability of measurement are the major concerns (29, 30). Although it is ideal for measuring overall repair of DNA damage, such as photoproducts or adducts (18), the HCR assay may not be applicable to damaged plasmids with single-strand or double-strand breaks that reduce plasmid transfer frequency (31), not allowing to differentiate between repair-deficient cells and normal ones. Therefore, in contradiction to other reports (32, 33), the HCR system is not suitable for evaluating cellular repair of DNA strand breaks.

Compared to the HCR assay, the mutagen sensitivity assay has some limitations in terms of confounding effects of cytotoxicity of the test agents, but it can accommodate more test agents with various genotoxicities. Therefore, we conclude that it would be more informative to use a battery of complementary assays in measuring the cancer susceptibility of an individual at different levels (e.g., DNA and chromosome) in future epidemiological studies. The findings in this study warrant further studies to verify the correlation between DNA repair capacity and mutagen sensitivity in the general population. The relationship of DNA repair capacity and mutagen sensitivity in human cells and their interactive role in cancer etiology needs to be further investigated in future molecular epidemiological studies of cancer.

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