Epidemiology of Ultraviolet–DNA Repair Capacity and Human Cancer

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The following conclusions are derived from a epidemiological study. Reduced repair of ultraviolet (UV)-induced DNA damage contributes directly to basal cell carcinoma (BCC) in individuals with prior sunlight overexposure. A family history of BCC is a predictor of low DNA repair. Repair of UV-damaged DNA declines at a fixed rate of approximately 1% per annum in noncancerous controls. The DNA repair differences between young BCC cases and their controls disappear as they age. Hence, BCC, in terms of DNA repair, is a premature aging disease. The persistence of photochemical damage because of reduced repair results in point mutations in the p53 gene and allelic loss of the nevoid BCC gene (Gorlin’s syndrome) located on chromosome 9q. The fact that environmental vulnerability is gender oriented implicates hormones in regulating DNA repair. Xeroderma pigmentosum appears to be a valid paradigm for the role of DNA repair in BCC in the general population. — Environ Health Perspect 105(Suppl 4):927–930 (1997)

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Introduction

Human populations typically display a range of inherent sensitivities to radiation and chemical carcinogens. Such variability in host response may be due in part to inherent differences between individual ability to monitor and repair damaged sites induced in their genetic material by exogenous and endogenous genotoxic agents. A human model supporting such an assumption exists with the rare, cancer-prone inherited disorder xeroderma pigmentosum (XP) (1,2). XP patients experience a greater than 1000-fold excess frequency of sunlight-related skin cancers. Coupled to this marked susceptibility is the consistent laboratory finding that all cells tested from XP patients are defective in repairing DNA damage induced by ultraviolet (UV) radiation and other UV-mimetic agents. Because the UV component of solar radiation exists as the predominant environmental risk factor for skin cancer, a causal association between UV exposure, defective repair of UV-induced DNA photoproducts, and skin cancer is inferred. The link is further strengthened by clinical reports showing that the occurrence of skin tumors is practically ameliorated in XP patients afforded early and lifelong protection from sunlight exposure (3,4).

Taken together, these observations show that DNA repair capacity (DRC) could exist as an etiologic correlate of cancer risk outside of XP. If XP is considered to represent the lower range of repair capabilities in humans, those individuals expressing a somewhat reduced repair response within the upper quadrant or shoulder of a dose–response curve (hallmark of repair) may be at increased risk for skin cancer or internal neoplasms given an appropriate exposure. Because it can be anticipated that human populations may include individuals who show only marginal damage vulnerability, any repair assay must be able to detect the level of DRC found in the heterozygotes of autosomal recessive DNA repair diseases with extreme precision and minimal intraassay variation.

The extent to which this hypothesis may be evaluated is dependent on the availability of validated laboratory methodology with which to measure DNA repair proficiency within study populations of interest. Such laboratory methodology has been developed and validated with over 450 subjects in case–control studies.

Methods and Results

T-lymphocytes isolated from subjects’ peripheral blood were assayed (Figure 1) for their DRC (5). The assays used plasmid DNA containing photoproducts resulting from three UV doses and were carried out in triplicate for each dose from 0, 350, to 700 J/m². The results are reported as the percent of cat gene expression (% CAT activity) following repair of damaged DNA compared to undamaged plasmid DNA. Lymphoblasts from patients with XP-A (Group A, most severe), XP-D (Group D, severe), and XP-C (Group C, classic form) provided the standard DNA repair of known levels of deficiency curves. Also, lymphoblasts from normal individuals (GM0131 and GM1892) were included in the standard normal repair curves. The DRC measurements obtained at UV doses of 700 J/m² (26 pyrimidine dimers per DNA molecule) and 350 J/m² generated straight line functions. Under these circumstances only the measurements at a dose of 700 J/m² were used for group comparisons.

The participants consisted of 88 cases with primary basal cell carcinoma (BCC) as diagnosed through the dermatopathology laboratory of the Johns Hopkins Hospital, which serves multiple practicing dermatologists in Maryland. The 135 comparison controls had skin biopsies for diagnosis of mild skin disorders such as seborrhic keratoses, intradermal nevus, or subacute eczematous dermatitis. All subjects were between 20 and 60 years of age, lived in Baltimore City or its suburban area for most of their lives, and had skin biopsies in 1987 to 1990. The purpose of selecting only young subjects was to maximize the difference in risk factor between cases and controls. At a clinic visit, dermatologists examined all participants to classify skin type and describe current skin conditions. The subjects then gave written informed consent, completed a structured questionnaire, and provided blood. Control individuals with a self-reported history or clinical signs of skin cancer or other cancers were excluded.
Low DNA Repair Capacity as a Risk Factor

In general, individual DRC values varied in this population. Compared to controls, the DRC of cases was shifted to the low end of the range (Figure 2). The cases had a mean DRC (7.4%) lower than that of the controls (7.8%). This difference observed was somewhat short of being statistically significant with a p value of 0.097. In previous reports (5,6) 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 30th percentile of the controls had a greater than 2-fold increased risk for BCC [odds ratio, 2.3; 95% CI, 1.2-4.5; adjusted for age (based on two-sided Student's t test)].

Age Stratification

The DRC of the subjects declined with age over the 20- to 60-year span studied (6). This age-related decline (apoptosis) occurred in both cases and controls, although it only reached significant decline in the controls (Figure 3). In the 106 controls, lacking any family history of skin cancer or presence of any premalignant skin lesions, the decline is 0.63% per year between 20 and 60 years of age. This would amount to about a 25% decrease in cumulative DRC over a 40-year period.

This age-related decline in DNA repair should be accompanied by an increased accumulation of persistent DNA damage affecting an increase in mutation fixation in structural and functional proteins. The mutation rate accompanying aging in the hprt gene in human lymphocytes is reportedly between 1.3 and 1.6% per year (7), twice the rate of decline of DNA repair reported here. Many known genetically linked repair-deficiency diseases, such as Cockayne's syndrome and XP, also manifest premature aging (8). In XP patients, the defect in DRC is associated with age-related skin changes and development of skin cancer 20 years earlier than in the normal population (2). Other workers have reported age-related changes in unscheduled...
DNA synthesis (UDS) in lymphocytes (9,10) and epidermal cells (from blood donors, especially in the aged). These findings are consistent with the age-related decline in DRC observed in this study.

We found an age-related decline in post-UV DRC of −0.6% per year in cultured primary skin fibroblasts from normal donors from the first to the tenth decade of life. There was a corresponding age-related increase in post-UV mutability measured as mutations into transfected UV-treated plasmid (pSP189) of +0.6% per year in lymphoblastoid cell lines from normal donors of the same age range. This study indicates that aging in humans is associated with decreasing ability to process new UV-induced DNA damage and this age-related reduction in DRC and increase in mutability is reflected in cultured skin and blood cells (11).

Similar to the XP model, BCC cases with first skin cancer at an early age repair DNA photoproducts poorly compared to controls or with cases expressing BCC at a later age of onset. This suggests that poor DNA repair is associated with the precocious aging manifested by an early onset of BCC. After adjusting for age at onset, the age-related decline in the repair of UV damage among the cases was at least as sharp as that of controls after adjustment for the age at onset of first cancer. After controlling for current age, the age of onset of BCC was positively correlated with DNA repair.

Familial History
Several findings suggest that the early age of onset of skin cancer and the reduction of DRC have familial links. Control subjects with a family history of BCC or with actinic keratoses had low DNA repair levels similar to those of the cases. After removing these positive controls, the overall difference in DNA repair between cases and controls was statistically significant.

Among those cases between the ages of 20 and 44 having BCC (n = 38), about 45% had a family history of BCC whereas only 10% of cases who had BCC at ages 55 to 60 (n = 21) had a similar history. This trend was statistically significant. In contrast, only 16% of controls (n = 135) had a previous skin cancer history.

Multiplicity of Tumors
Multiple linear regression models were used (Figure 4) to correlate DRC with the number of skin cancers (least-square estimate of regression coefficient from multiple linear regression models). The estimated odds ratio was used to describe the risk of BCCs. The distribution of DRCs of the subjects was approximately normal, with a 5-fold variation between individuals. DRCs below the upper 30th percentile of controls were associated with an estimated 3.5-fold (95th CI, 1.17 to 4.54-fold) increased risk for the occurrence of BCCs. The lower the DRC, the greater the number of skin tumors in individuals (p < 0.05) after adjustment for age.

Sunlight Exposure
A fundamental question is whether the genotoxic–DNA repair paradigm of XP reflects the carcinogenic response of individuals who have relatively marginal reduction in DRC but have overconsumed this capacity with excessive sunlight exposure (Figure 5). In this case–control study, the proportion of cases (36%) that have been overexposed in their lifetime is double that of the controls (17%). The development of BCC, therefore, probably reflects the mutation fixation as a consequence of the persistent DNA damage resulting from individuals having exceeded their DRC. After UV exposure, subjects with a family history of skin cancer, possibly with reduced repair capacity compared to others of the same age, will develop this disease at an early age. It is implied in subjects displaying a time-delayed onset of BCC that mutation fixation may also occur as a result of accumulated, excessive unrepair DNA damage in individuals who possess apparently normal DNA repair levels.

Gender Orientation
Stratification of the odds ratios according to gender (Figure 5) revealed the vulnerability to BCC of the skin of women who have a history of overexposure to sunlight and who have reduced repair capacity. These results agree with those obtained in the previous pilot study (5,6) in which young women who have a long-standing history of sunbathing were predisposed to BCC of the skin. This gender orientation suggests an explanation in terms of hormonal, genetic, or combined factors. The self-questionnaires included requests for information concerning stages of estrous, hormonal supplementation, and use of oral contraceptives. Surprisingly, postmenopausal women receiving estrogen supplementation had a significant increase in their DRC. These data show that hormones can regulate DRC, although they do not account for the inhibition of DNA repair; but the data provide important clues concerning agents that can turn on DNA repair. These data are reflected in the crossover of repair in Figure 3. When such age-stratified data are further stratified according to gender, the peculiar drop among the female BCC patients is seen among those who are premenopausal and not on estrogen supplementation, whereas the increase in repair characteristic of the postmenopausal female cases appears to reflect the large number of such women on estrogen supplementation.

Mutational Consequences of Persistent DNA Damage
If DNA repair is a primary target for apoptosis, we can anticipate 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. (7) of a concomitant increase in mutation fixation of a number of genes at the rate corresponding to an increase of 1 to 2% per annum over a wide age range.
The persistence of UV-induced photoproducts, attributed to reduced DNA repair, was also studied at the level of p53 genes in BCC (12). 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 nevoid BCC, or Gorlin's syndrome. Forty-four percent (16 of 36) of BCCs had a mutated p53 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.

Conclusions

The sunlight–BCC paradigm exemplifies the causal relationship between a specific kind of damage and the potential effects of the persistence of such damage in tumor progression; it also provides mechanistic insights into fundamental biological processes. These studies open doors for detailed examination of other forms of chromosomal instability in familial chronic diseases. Because the plasmid used in this DRC assay is a useful target for a large array of DNA-damaging agents it is feasible to extend these studies to a wide variety of diseases with potential genetic linkages. The only DNA damaging agents, in which this assay is not useful, however, are those that generate single- and double-strand breaks, since only supercoiled DNA is expressed in this assay system.

Many repair deficiency diseases, such as XP, are associated with neurodegenerative symptoms, suggesting relationships between repair and chronic diseases other than cancer. Certainly the age relatedness of many cancers may be a characteristic of deficient DRC or mismatch repair. Defective or deficient repair processes associated with aging require that population and case–control studies involving repair assure that age-matching is incorporated into study protocols. Further, it will be of great interest to determine which steps in the progression of DRC or mismatch repair are rate limiting, and to correlate organ specificity of a disease with specific steps in repair processes.

REFERENCES

1. Cleaver JE, Kraemer KH. Xeroderma pigmentosum. In: The Metabolic Basis of Inherited Diseases (Scriver CR, Beaudet AL, Sly WS, Valle E, eds). New York:McGraw-Hill International, 1989;2949–2971.
2. Lambert WC, Kuo H-R, Lambert MW. Xeroderma pigmentosum. Dermatol Clin 13:169–210 (1995).
3. Pawsey SA, Magnus IA, Ramsey CA. Clinical, genetic and DNA repair studies on a consecutive series of patients with xeroderma pigmentosum. Q J Med 48:179–210 (1979).
4. Lynch HT, Frichot BC, Lynch JF. Cancer control in xeroderma pigmentosum. Arch Dermatol 113:193–195 (1977).
5. Athas WF, Hedayati MA, Matanoski GM, Farmer ER, Grossman L. Development and field-test validation of an assay for DNA repair in circulating lymphocytes. Cancer Res 51:5786–5793 (1991).
6. Wei Q, Hedayati MA, Matanoski GM, Farmer ER, Grossman L. DNA repair and aging in basal cell carcinoma: a molecular epidemiology study. Proc Natl Acad Sci USA 90:1614–1618 (1993).
7. Cole J, Arlett CF, Green MHL, James SE, Henderson L, Cole H, Sala-Trepat M, Benzi R, Price ML, Bridges BA. Measurement of mutant frequency to 6-thioguanine resistance in circulating T-lymphocytes for human population monitoring. In: New Trends in Genetic Risk Assessment. London: Academic Press, 1989;175–203.
8. Guzzetta F. Cockayne–Neill–Dingwall syndrome. In: Handbook of Clinical Neurology, Vol 13 (Vinken PJ, Bruyn W, eds). Amsterdam:Biomedical Press, 1972;421–440.
9. Matin MG. Genetic syndromes in man with potential relevance to the pathology of aging. In: Genetic Effects on Aging (Bergsma D, Harrison DH, eds). New York:Liss, 1978;5–39.
10. Lambert B, Ringborg U, Skoog L. Age-related decrease of ultraviolet light-induced DNA repair synthesis in human peripheral leucocytes. Cancer Res 39:2792 (1979).
11. Moriwasaki S-I, Ray S, Tarone RE, Kraemer KH, Grossman L. The effect of donor age on the processing of UV-damaged DNA by cultured human cells: reduced DNA repair capacity and increased mutability. Mutat Res 364:117–123 (1996).
12. van der Riet P, Karp D, Farmer E, Wei Q, Grossman L, Tokino K, Ruppert JM, Sidransky D. Progression of basal cell carcinoma through loss of chromosome 9q and inactivation of a single p53 allele. Cancer Res 54:25–27 (1994).