Familial Melanoma Associated with Dominant Ultraviolet Radiation Sensitivity

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ABSTRACT

Sensitivity to ultraviolet radiation was studied in lymphoblastoid cell lines derived from 32 members of two families with histories of multiple primary melanomas in several generations. As assayed by colony formation in agar or by trypan blue exclusion following irradiation, cellular sensitivity showed a bimodal distribution. All persons with melanoma or multiple moles were in the sensitive group, while some family members exhibited responses similar to those of controls. Cells from four cases of sporadic melanoma showed normal levels of sensitivity. The data are consistent with a dominantly inherited ultraviolet light sensitivity associated with these examples of familial melanoma.

Spontaneous and ultraviolet light-induced sister chromatid exchange frequencies were similar to those in control cell lines. No defect in excision repair was detected in any of the above cell lines, but the sensitive group showed postirradiation inhibition of DNA replication intermediate between controls and an excision-deficient xeroderma pigmentosum cell line.

INTRODUCTION

The accumulated evidence from studies on XP3 in the past decade (20) demonstrates that autosomal recessive mutations can be important determinants of some cases of malignant melanoma in humans. Evidently, homozygosity is required, since epidemiological evidence suggests that XP heterozygotes are not at increased risk of developing melanomas (22). While the majority of melanomas are sporadic, a proportion occur in successive generations of a family. A population-based study in Australia (25) estimated that first-degree relatives of melanoma patients were 1.7 times more likely to develop melanoma than were persons in the general population. In this study, it was estimated that 11% of malignant melanoma in that population was of genetic origin. It has been pointed out that the latter figure is likely to be an underestimate because of the high UV exposure in the population studied, which would be expected to increase the rate of sporadic cases (10). However, in general, some of the patterns observed do not necessarily exclude familial clustering on the basis of common environmental exposure.

Delineation of genetic and environmental factors is important both in the elucidation of the etiopathogenesis of this class of malignant tumor and for prognosis of individuals within families in which melanoma occurs. The description of the B-K mole or dysplastic nevus syndrome involving heritable melanocytic lesions which predispose to familial malignant melanomas (4) has been an important contribution to the definition of genetic factors predisposing to melanoma. We report here laboratory data, from studies on 2 large unrelated pedigrees with multiple cases of malignant melanoma, which are consistent with a dominant mutation giving rise to UV radiation sensitivity in association with a liability to develop cutaneous malignant melanoma.

MATERIALS AND METHODS

Cells. Peripheral blood lymphocytes were isolated (16) from 12 members of Family A (designated in the series FM201ABR-FM214ABR) and 20 members of Family B (designated in the series FM101ABR-FM136ABR). Family A members were of Celtic origin, living in a district about 100 miles southwest of Brisbane. There were 6 known cases of cutaneous malignant melanoma among 13 individuals in 2 generations (generations I and II). Some individuals also had basal cell carcinomas and/or moles. Five members in generation II and 7 of their children (generation III) were available for study. Family B members were also of Celtic origin, living in or near Perth in Western Australia and unrelated to Family A. Fourteen cases of cutaneous malignant melanoma were recorded among 30 members in 3 generations (generations I, II, and III). Some members had dysplastic nevi, but neither family conformed in all respects to the B-K mole syndrome. Lymphocytes were also isolated from 4 sporadic cases of melanoma (cell lines 371LABR, 373LABR, 376LABR, and 377LABR) with no family history of the disease. Nine control samples (designated in the series C1ABR-C11ABR) were collected from individuals with no personal or family history of melanoma or other skin cancer, and one XP cell line (XP301ABR) was established from a patient with neurological abnormalities (Complementation Group A). Lymphocytes were transformed with Epstein-Barr virus to produce lymphoblastoid cell lines (15). An XP variant (GM2449) cell line was obtained from the Human Genetic Mutant Repository, Camden, N. J. Cells were grown in RPMI 1640 (Microbiological Associates, Walkersville, Md.) medium supplemented with 16% fetal calf serum at 37° in 5% CO2.

UV Irradiation. Cells were washed and suspended in PBS. A thin film of the cell suspension of up to 105 cells/ml was added to a 35-mm bacterial plate and irradiated with a UV lamp (Mineralite UVS 12; Ultraviolet Products, Inc., San Gabriel, Calif.), at a dose rate of 0.5 J/sq m/sec. The incident dose of UV was measured with a UV intensity meter (Ultraviolet Products, Inc.), or by malachite green spectrophotometry (9). Medium was returned to cultures immediately after irradiation.

Determination of UV Sensitivity. Cell survival after UV irradiation was determined by colony formation in agar (2). The viability of lymphoblastoid cells at various times after UV irradiation (15 J/sq m) was determined with 15% fetal calf serum at 37° in 5% CO2.

DNA Repair Synthesis. Lymphoblastoid cells growing exponentially at a density of 5 × 105/ml were incubated with 20 mM hydroxyurea
scored for each treatment.

Cooled and stained with Giemsa (Gurr, Hopkin and Williams) in phosphate buffer (pH 6.8) (24). Twenty-five or more metaphase cells were irradiated with UV (2 or 4 J/sq m) and Family B (4.4 J/sq m).

However, the mean D₀ values of sensitive groups (Group S) in Family A (2.4 J/sq m) and Family B (2.9 J/sq m) were significantly lower than those of both control and normally sensitive family members (p < 0.001, by the Wilcoxon-Mann-Whitney rank-sum test).

Individuals in Families A and B affected by melanoma are indicated in Charts 3 and 4, respectively. Those individuals considered to be abnormally UV sensitive are marked S, and normally sensitive individuals are marked N. In both families, no Group N individual had melanoma; all individuals with melanoma or multiple moles belonged to Group S. Sensitivity bore no Group N individual had melanoma; all individuals with melanoma or multiple moles belonged to Group S. Sensitivity bore

RESULTS

UV Sensitivity. Lymphoblastoid cell lines have been shown to reflect UV sensitivity exhibited by fibroblasts in the case of XP (1) and ionizing radiation sensitivity in the case of ataxia telangiectasia (2). In the present experiment, irradiated lymphoblastoid cells showed a bimodal distribution of radiosensitivity in both families. Data obtained by assay of prolonged viability by trypan blue exclusion for cell lines listed from Family A (not shown) were consistent with data obtained with the same cell lines assayed by colony formation in agar (Chart 1). Both methods indicated that there was an abnormally UV-sensitive group (Group S) and a normally sensitive group (Group N).

In view of the identical subgroup classification of all individuals in Family A by both methods, cell lines from Family B and 4 sporadic melanoma patients were assayed for UV sensitivity by the preferred method of colony formation only. Chart 2 shows the distribution of D₀ values obtained in these experiments. Family B may also be divided into 2 distinct groups.

The mean D₀ value for the control cell lines was 4.5 J/sq m. This value was not significantly different to those for the sporadic melanoma cell lines (4.3 J/sq m) or the normally UV-sensitive groups (Group N) in Family A (4.2 J/sq m) and Family B (4.4 J/sq m).

Chart 1. Survival of lymphoblastoid cell lines following UV irradiation measured by colony formation in agar. ◆, controls; ▲, XP-A; ▐, Family A Group N; □, Group S without melanoma; ▌, Group S with melanoma. Each symbol represents the mean of measurements on a separate cell line.

Chart 2. D₀ values calculated from plotted clonal survival curves of lymphoblastoid cell lines. A, normal controls; B, sporadic melanoma patients; C, Family B Group N (□) and Group S without (■) and with (●) melanoma.
no relation to age. In both groups, there was more than one sensitive member of each generation.

DNA Repair Synthesis. Following UV irradiation, DNA repair synthesis in UV-sensitive Group S cell lines [5.90 ± 1.22 (S.E.) \times 10^4 \text{ cpm/10^6 cells}] was similar to that carried out in controls [4.32 ± 1.72 \times 10^4 \text{ cpm/10^6 cells}] and Group N lines [5.56 ± 3.76 \times 10^4 \text{ cpm/10^6 cells}]. There was no evidence of a deficiency in repair synthesis as is seen in excision-deficient XP cells [4.10 ± 3.8 \times 10^3 \text{ cpm/10^6 cells}].

Unscheduled DNA Synthesis. The level of incorporation of [3H]thymidine in the non-S-phase cells was found to be similar in Group S cells and control cells. The mean number of grains per nucleus was significantly higher than background for these 2 cell lines, attesting to the presence of active unscheduled DNA synthesis. The mean number of grains per nucleus in the case of XP301 was not significantly different from background as has been reported in studies on XP-A cell lines (11).

Inhibition of DNA Replication. Following UV irradiation, DNA replication is inhibited in excision-deficient and variant XP cells, in Cockayne syndrome cells, and in the UV-sensitive cell line 11961 (5, 14, 21). The rates of DNA replication in representative normal, Group S, and XP cells after UV irradiation, compared with unirradiated controls, are shown in Chart 5. In the UV-sensitive cell lines FM136 and FM210, DNA synthesis was depressed to a minimum 2 hr after irradiation with 5 J/sq m and increased slowly to the levels in unirradiated samples of the same cell lines, after approximately 6 hr incubation; the same was true for all Group S cells tested (not shown). The XP301 cells showed a more severe depression of synthesis and failed to recover in the time of the experiment. DNA synthesis in C11 cells was slightly depressed but recovered to normal levels after approximately 4 hr incubation; the same was true for other control cells and for cells from Group N.

UV Sensitivity in Familial Melanoma

The rate of DNA chain elongation in Group S cells following 12.5 J/sq m was indistinguishable from that in control cells and Group N cells, while XP variant GM2449 cells showed the characteristic delay expected of this mutant class (13) (data not shown).

SCN. The frequency of SCEs per cell induced in presence of BrdUrd alone or UV plus BrdUrd was determined. The spontaneous or BrdUrd-induced frequencies of SCEs found in all cell lines examined were low, in the range reported for Epstein-Barr virus-transformed lymphoblastoid cell lines, at 3 to 5 SCEs/cell (3, 24). Following UV irradiation with either 2 or 4 J/sq m, examined cells showed a dose-dependent increase in SCE frequencies above the spontaneous level. This was particularly evident in the case of XP301. No significant differences were observed between representative controls (7.68 ± 0.49), GM2449 (9.03 ± 0.86), and UV-sensitive cell lines (8.76 ± 0.69) from both families when UV-induced SCE frequencies were compared at a dose of 4 J/sq m. At this dose, XP301 cells gave 20.88 ± 1.68 SCEs/cell. These data are consistent with those published for XP-A, normal and XP variant lymphoblastoid (3), and fibroblast (7) cell lines. All cells examined were essentially diploid.

DISCUSSION

In the families described here, the pattern of occurrence of melanoma (Charts 3 and 4) is consistent with dominant inheritance and incomplete penetrance, a pattern frequently observed in familial melanoma (10). The pattern of UV sensitivity in these families is also consistent with dominant inheritance. Since the cells of those family members examined who had melanoma fell into the UV-sensitive class, it is possible that UV sensitivity directly confers a predisposition to the development of melanoma. However, the observation that some UV-sensitive individuals in the appropriate generations of both families do not have melanoma must be accounted for. This may simply reflect a latency period such that these UV-sensitive individuals...
will later develop melanoma or that an environmental factor which triggers the development of melanoma in sensitive individuals has not been encountered.

The association of UV sensitivity with melanoma could be accounted for by proposing that the UV-sensitive gene is closely linked to a hypothetical melanoma gene if we assume that the underlying defect in lymphoblasts is also present in melanocytes. Where UV sensitivity is found in individuals who do not have melanoma, the explanation may rest with time, chance, or chromosomal cross-over in that branch of the family, disassociating the UV sensitivity and melanoma genes. Conceivably, UV may act as a promoter in the genesis of melanoma in UV-sensitive family members, with the initiation step being provided by the melanoma gene. Other factors subject to genetic control and inheritance in these families and involved, for example, in the immune system (6) or endocrine system (17) may modify the development of melanoma in individuals carrying the UV sensitivity gene.

In general, UV sensitivity is not a prerequisite for the development of melanoma since the lymphoblastoid cell lines from a number of cases of sporadic melanoma were no more sensitive to the lethal effects of UV radiation than were normal cells. The individuals with melanoma in the 2 pedigrees studied were different from sporadic cases in this respect.

The nature of the defect in the UV-sensitive familial melanoma cell lines remains to be elucidated. The normal level of DNA repair synthesis, measured autoradiographically or by scintillation counting, makes it unlikely that there is an excision repair deficiency such as that seen in XP. Normal repair synthesis has been reported previously in cell lines of a number of melanoma patients (19). The finding of normal rates of DNA chain elongation following irradiation appears to exclude mutants of the XP variant type. However, the recovery of DNA replication in Group S cells after irradiation was delayed compared with normal controls. These data together suggest that Group S cells initiate fewer replicons following UV irradiation than do normal cells. The possibility that UV sensitivity might confer altered liability to UV-induced mutagenesis needs to be explored.

The finding of a dominant UV sensitivity in association with familial melanoma serves to stimulate further the study of genetic factors underlying susceptibility to this type of tumor. This observation does not imply that all familial melanomas will exhibit such an association; indeed, we would be surprised if this were so. However, a detailed examination of this group of mutants at a molecular level should contribute to the characterization of genetic factors involved in the genesis of melanoma and perhaps may ultimately be of prognostic value within certain families.

REFERENCES

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