Differential Behaviors toward Ultraviolet A and B Radiation of Fibroblasts and Keratinocytes from Normal and DNA-Repair-deficient Patients

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ABSTRACT

Xeroderma pigmentosum (XP) and trichothiodystrophy (TTD) are rare genodermatoses transmitted as recessive and autosomal traits that result in reduced capacity to repair UV-induced DNA lesions. Although XP, but not TTD, patients are prone to basal and squamous cell carcinomas, to date no comparative studies of the XP and TTD phenotypes have included epidermal keratinocytes. We compared the DNA repair capacity (by unscheduled DNA synthesis) and cell survival (by clonal analysis) of epidermal keratinocytes and dermal fibroblasts grown from normal individuals and patients with xeroderma pigmentosum and trichothiodystrophy following UVA and UVB irradiation. The same dose of UVB (1000 J/m²) induced twice as many DNA lesions in normal fibroblasts as in normal keratinocytes. UV survival rates were always higher in keratinocytes than in fibroblasts. Normal and TTD keratinocytes survived better following UVA and UVB irradiation than XP-C and XP-D keratinocytes. XP-C keratinocytes exhibited exacerbated sensitivity toward UVA radiation. Unscheduled DNA synthesis at UV doses leading to 50% cell survival indicated that the ratio of DNA repair capacity to cell survival is higher in keratinocytes than in fibroblasts. In addition, UVA and UVB irradiation induced a transition from proliferative to abortive keratinocyte colonies. This transition varied between donors and was in part correlated with their cancer susceptibility. Altogether these data provide the first evidence of the differential behaviors of normal, XP, and TTD keratinocytes toward UV radiation.

INTRODUCTION

Skin cancers are the most frequent neoplastic afflictions in human beings. Their incidence increases with sunny climates and has been exacerbated dramatically by the increased enthusiasm in many countries for the beach and suntans. The large majority of skin cancers can be divided into melanomas and nonmelanoma neoplasms. Two types of nonmelanoma skin cancers are known, BCC and SCC. BCCs and SCCs develop from epidermal keratinocytes, whereas melanomas originate from melanocytes. These cells are the primary cellular targets of solar UV radiation that reaches the earth's surface (UVB, 290–320 nm, and UVA, 320–400 nm). UVA and UVB wavelengths above 300 nm reach the basal epidermal layer, which contains keratinocytes with high proliferative potential (4), and fibroblasts located in the underlying dermis.

The potential catastrophic effects of UV radiation are mimicked in some hyperphotosensitive genodermatoses, such as XP. XP is a rare (~1 in 250,000) autosomal disease characterized by a highly increased (2000-fold) predisposition for cancer in sun-exposed areas (9). Seven XP complementation groups have been identified (groups A to G). A variant form of XP called XP variant (XP-V) has also been described. The genes corresponding to groups XP-A through XP-G have been cloned (10, 11). Interestingly, mutations in the XPD gene and in XBP gene are also responsible for another hyperphotosensitive genodermatosis, TTD, which lacks the predisposition for skin cancer observed in XP patients (12, 13). All of the XP gene products are involved in specific steps of the DNA-repair process that removes UV-induced DNA lesions by a mechanism called NER (11). Lesions generated by short UVB wavelengths and by UV-C (14) are mostly CPDs and 6-4 PPs. In the absence of efficient DNA repair, e.g., in XP cells, these lesions persist in the genome and may lead to the introduction of deleterious mutations after DNA replication. Molecular epidemiology studies have clearly demonstrated that ~50% of epidermal tumors harbor DNA mutations in the p53 tumor suppressor gene at bipyrimidine sites that are characteristic of UV irradiation (i.e., mostly CC→TT tandem mutations and C→T transitions; 15). Furthermore, in vitro experiments using shuttle DNA reporter vectors transfected in fibroblasts from XP patients have established clearly the highly mutagenic and distinctive properties of UV-induced DNA lesions (16, 17).

To date, almost all efforts devoted to the characterization of the XP phenotype have been focused on the molecular mechanisms of DNA repair itself rather than on the physiological response of epidermal cells after UV irradiation. Paradoxically, most studies have been performed using dermal skin fibroblasts, but very few have included the study of DNA repair properties and UV survival of epidermal keratinocytes, those cells from which epidermal cancers originate. To address this question, we first collected both epidermal keratinocytes and dermal fibroblasts from patients with either XP or TTD. The responses of these cells to UV radiation were assessed in parallel by clonal analysis and by measurement of DNA repair properties after exposure to UVA and UVB ranges.

Our data integrate crucial parameters that participate in the development of UV-induced epidermal tumors.

MATERIALS AND METHODS

Patients. The XP-C (XP148VI cells) patient had developed multiple skin tumors beginning at the age of 9 years. Surgical excision of eight BCCs and six SCCs in sun-exposed areas were documented. The complementation group was determined as XP-C in our laboratory by the heterodikaryon complementation test. The XP-D patient (XP22VI cells) had developed four BCCs and one SCC in sun-exposed areas and suffered from melanosomes as well. The patient’s age when the first skin tumor appeared was 12 years. The complementation group was determined as XP-D, and the mutation in the XPD gene in XP22VI cells was determined as R683W (18).

TTD (TTD13VI) cells used in this study were obtained from the back skin of a fetus expelled at an estimated gestation age of 29 weeks. Both parents were heterozygous for the XP-D gene. A previous child from the same parents was also diagnosed clinically as TTD (TTD2VI) and died at the age of 14 years. Another fetus diagnosed as TTD was also aborted. The antenatal diagnosis of TTD (TTD13VI) cells used here was established by UDS analysis of amniocytes and by the observation of characteristic alternate bands of dark and bright areas in fetal hair using polarizing microscopy; the diagnosis was confirmed after expulsion by the microscopic examination of skin and of scalp.

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2 The abbreviations used are: BCC, basal cell carcinoma; SCC, squamous cell carcinoma; XP, xeroderma pigmentosum; TTD, trichothiodystrophy; NER, nucleotide excision repair; CPD, cyclobutane pyrimidine dimer; 6-4 PP, 6-4 pyrimidine-pyrimidone photoduct; UDS, unscheduled DNA synthesis; CFE, colony-forming efficiency; mab, monoclonal antibody; WT, wild type; LSP, large with smooth perimeter; ST, small, highly irregular, and terminal; W, wrinkled.

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Cell Culture. Biopsies from nonexposed skin areas (groin or buttock) were obtained after approval was received from adult subjects or the parents of minors. Fibroblasts and keratinocytes were derived from the same donors except for normal cells. Normal fibroblasts were obtained from baby foreskin (age, 18 months). Keratinocytes were obtained from abdominal surgery of a 3-year-old baby.

Human epithelial keratinocytes of normal, XP, and TTD individuals were obtained and cultured as described by Rheinwald and Green (19) on a feeder layer of X-ray-irradiated (60 Gy) Swiss 3T3 fibroblasts.

Human diploid fibroblasts were grown in DMEM medium (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS (Dominique Dutschker, Mulhouse, France), 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 10,000 IU of penicillin-streptomycin, and 2 mM L-glutamine for 100 ml of culture medium.

Cells were cultured at 37°C in a 9.5% CO₂ atmosphere. All experiments were performed using keratinocytes and fibroblasts at passages 3 to 5.

UV Radiation Sources. UVA and UVB, and UVC irradiation were performed as described previously and using the same materials (2, 20).

UV Cell Irradiation. Irradiation was performed on 60–70% confluent cells. The cells were first rinsed twice with PBS prewarmed at 37°C and irradiated under a film of PBS for UVA irradiation and without PBS for the short periods needed for UVB. To avoid excessive warming of the cells during UVA irradiation, dishes were placed on a cooling metal plate with an inside flow of temperate water to maintain cells at 37°C.

Determination of Cell Survival. For clonal analysis of keratinocytes, cells were grown in 60-mm Petri dishes for ~5 days and then irradiated under UVA (doses ranging from 100,000 to 500,000 J/m²) or UVB (100 to 1200 J/m²). Immediately after irradiation, cells were trypsinized (in 0.05% trypsin, 0.01% EDTA) and counted. Keratinocytes were seeded at clonal density (35 cells/cm²) onto previously irradiated 3T3 Swiss fibroblasts. Cultures were maintained in keratinocyte medium containing 10% FCS (BioMedia, France). From the fourth day after plating, the medium was supplemented with epidermal growth factor at 10 ng/ml (Euromedex, France). The culture medium was changed every 4 days. After 12 days, keratinocyte colonies were fixed in 3.7% formaldehyde in PBS and colored by 1% rhodamine B (Sigma Chemical Co., St. Louis, MO). The numbers of colonies were determined by eye and under binoculars to ensure that all colonies, including small, abortive colonies, were counted. The relative cell survival was calculated as the number of colonies obtained after UV irradiation per total colony number obtained from unirradiated cells × 100. The CFE was between 7.5 and 10.5%. The three types of keratinocyte colonies were quantified according to the criteria given in the “Results.”

The colony-forming ability of fibroblasts after irradiation was determined by seeding increasing cell numbers as a function of UV doses from 1 × 10⁴ to 8 × 10⁶ cells per 100-mm dish as described (2). Cells were maintained in culture for 14 days. Cell culture medium was changed once at day 8 after seeding. Fibroblast colonies were fixed in a mixture of methanol/formaldehyde (9:1, v/v) and were stained by crystal violet. The relative survival was calculated as for keratinocytes. The CFE was between 9.5 and 10.5%.

At least two independent experiments were performed in duplicate dishes for each experimental point.

UDS Analysis. Keratinocytes and fibroblasts were seeded on glass coverslips, and DNA repair after UV irradiation was followed by [³H]thymidine incorporation as described previously (21) except that hydroxyurea and fluorodeoxyuridine were both omitted. UDS of keratinocytes was performed in keratinocyte culture medium containing 1% dialyzed FCS. UDS of fibroblasts was performed in DMEM supplemented with 1% dialyzed FCS. Coverslips with cells were mounted onto glass slides dipped in Amersham EM-1 photoemulsion. The exposure time was 1 week at 4°C for keratinocytes and 3–4 days for fibroblasts. In control experiments, UDS of both cell types was performed using the same culture medium (DMEM or F10 medium), the same dialyzed FCS (Dominique Dutschker), and the same exposition time (72 h) at 4°C. In all cases, the mean number of grains per nucleus was obtained by counting 80 non-S phase nuclei for each UV dose. All UDS experiments were performed at least twice. All experimental points were made in duplicate dishes.

Quantitative Determination of the Major UV-induced DNA Lesions, CPDs, and 6–4 PP in Genomic DNA. Exponentially growing fibroblasts and keratinocytes were UVB-irradiated at a dose of 1000 J/m². Following irradiation, DNA was prepared as described previously (20). Purified DNA was loaded on PVDF membrane (Hybond-P; Amersham, Braunschweig, Germany). Membranes were then immersed in PBS containing 5% low-fat dried milk and 0.1% Tween 20 for 1 h at room temperature and washed in the same buffer containing the specific mabs directed either against 6-4 PP (6-4 M-2 mab) or CPDs (TDM-2 mab) for 1 h at 37°C in an orbital shaker. The TDM-2 mab was diluted to 1:2000; the 6-4 M-2 mab was diluted to 1:500. Chemiluminescent detection was performed using the ECF Western blotting kit (Amersham). The secondary antibody was linked to FITC, the tertiary anti-FITC antibody was linked to the alkaline phosphatase enzyme. Quantification of signals was obtained after scanning the blots on a FluorImager (Molecular Dynamics, Sunnyvale, CA) using the ImageQuant software (Amersham).

Statistical Study. Means were compared using the Mann-Whitney and Student t tests. A result was considered as not significant when its associated P was >0.05.

RESULTS

DNA Repair Capacity and Quantitative Assessment of UV-induced DNA Lesions

DNA repair by NER of fibroblasts and keratinocytes from normal, XP-C, XP-D, and TTD/XP-D were measured by UDS (Fig. 1a) following irradiation of cells with increasing doses of UVA and UVB, and also UVC wavelengths as a control. Irradiation with UVA doses up to 500,000 J/m² did not result in a grain number above the background in any cell strain. Data obtained after irradiation with increasing doses of UVB and UVC are presented in Fig. 1b and show that the increase of grain numbers in normal cells was virtually linear. The lowest UDS values were found in XP-C (~10% of those from WT cells and in XP-D (~20% of those from WT cells). Intermediate UDS values were found in TTD/XP-D fibroblasts significantly (~0.01) higher than those measured in the corresponding keratinocytes. This observation suggested that either fewer lesions were present in keratinocytes or that repair of lesions was more efficient in fibroblasts than in keratinocytes. To decide which of these hypotheses was correct, UV-induced DNA lesions (6-4 PP and CPDs) present in the DNA from keratinocytes and from fibroblasts were quantified by immunoblotting using specific antibodies against these lesions (22, 23). Fig. 2 shows that the number of both 6-4 PP and CPD DNA lesions in keratinocytes is ~50% of that found in fibroblasts for a UVB dose of 1000 J/m².

Cell Survival Measurement As the CFE after UV Irradiation

In the absence of UV irradiation, the CFE of fibroblast and of keratinocyte strains ranged between 7.5 and 10.5%.

Fibroblasts

Nonconfluent cultures of fibroblasts from normal, XP-C, XP-D, and TTD/XP-D were irradiated with either UVA or UVB wavelengths. Cells were then dissociated and plated at increasing densities according to the UV dose. This procedure was used because irradiation using increasing doses of UV of fibroblasts seeded at constant densities did not give enough colony for reliable counting and the statistical analysis of results.

UVB Wavelengths. Variable ranges of UV irradiation comprising four doses were determined by preliminary experiments according to the UV sensitivity of each strain (Fig. 3a). Fig. 3b (panel D) shows that irradiation of all fibroblast strains induced a dramatic decrease of cell viability. Normal fibroblasts exhibited much higher resistance to UVB irradiation than XP or TTD cells. The UVB doses leading to 50% cell survival for the fibroblast strains are indicated in Table 1.

UVA Wavelengths. All fibroblast strains were thus exposed to either three or four doses ranging from 50,000 to 500,000 J/m² (Fig. 3b, panel C). At doses below 150,000 J/m², the four cell survival...
curves were very close. The differences in UV sensitivity between normal cells and TTD/XP-D, XP-C, and XP-D cells was much more marked for higher UVA doses than for low (100,000 J/m²) or intermediate (250,000 J/m²). In the same dose range, the resistance of XP-C keratinocytes was significantly (P < 0.001) lower than that of other strains. The UVA doses leading to 50% cell survival for the keratinocyte strains are indicated in Table 1.

UV Wavelengths. All keratinocyte strains were exposed to three irradiation doses from 100,000 J/m² up to 500,000 J/m². The survival curves of normal, TTD/XP-D, and XP-D keratinocytes were very similar for UV doses ≤250,000 J/m². In the same dose range, the resistance of XP-C keratinocytes was significantly (P < 0.001) lower than that of other strains. The UVA doses leading to 50% cell survival for the keratinocyte strains are indicated in Table 1.

UV Irradiation Differentially Influences the Types of Colonies in Normal and in Repair-deficient Keratinocyte Cultures

Epidermal keratinocyte cultures grown at clonal density on lethally irradiated 3T3 cells give rise to three types of colonies: (a) colonies that are large (10–30 mm²) and have a smooth perimeter (LSP), which are rapidly growing colonies composed of cells with a proliferative potential expected from stem cells; (b) small (<5 mm²), highly irregular, and terminal (ST) colonies, which are founded by a cell with a short replicative life span, condemned to terminal differentiation; in these colonies all proliferation has ceased at the time of rhodamine staining (i.e., 12 days after seeding); and (c) wrinkled (W) colonies founded by a cell whose progeny has heterogeneous growth capacities, i.e., transitional between the two mentioned before. These three types of colonies (LSP, ST, and W) are initiated by cells called holoclone, paraclone, and meroclone, respectively. The discovery, description, and definition of holoclines, paraclones, and meroclines was proposed originally by Barrandon and Green (24).

The macroscopic and microscopic examination of culture plates showed evident variations in the morphology of keratinocyte colonies after UV irradiation. We thus quantified the relative variation of each type of colony obtained in each keratinocyte strain (Fig. 4).
Number of LSP and ST Colonies following UVA Irradiation

In three cell strains (WT, XP-D, and TTD/XP-D), the relative numbers of LSP colonies were slightly decreased and the relative numbers of ST colonies were increased after UVA irradiation. The relative number of both LSP and ST colonies did not vary significantly in XP-C keratinocytes exposed at a UVA dose.

Number of LSP and ST Colonies following UVB Irradiation

In three cell strains (WT, XP-D, and TTD/XP-D), the relative numbers of LSP colonies were significantly (WT, \( P < 0.05 \); TTD/XP-D and XP-D, \( P < 0.01 \)) decreased with a maximum in XP-D keratinocytes (84.2%). The decrease of LSP colonies was accompanied by the increase of ST colonies, with the highest relative value in XP-D keratinocytes (179.8%). In XP-C keratinocytes, UVB irradiation did not induce a significant change in the relative numbers of LSP or ST colonies.

Number of W Colonies after UVA or UVB Irradiation

Variations in the relative ratio of W colonies after UVA or UVB irradiation were observed. However, their amplitudes were much lower than those observed in the case of LSP and ST colonies. It was worth noting, however, that the highest relative increase of W colonies was observed using XP-C keratinocytes after both UVA and UVB irradiation (Fig. 4, C and D). In the three cases where the relative numbers of W colonies decreased (XP-D and TTD after UVB; XP-C after UVA), a significant (\( P < 0.01 \)) increase of ST colonies was observed.

Taken together, these data demonstrate that the relative ratio of LSP colonies...
Because high UVA doses (250 kJ/m²) induce tumors in rodents (34, 35) CPDs are hardly detectable by immunocytochemistry after a wide range lesions after UVA irradiation of human skin explants (33). Furthermore, is in agreement with the absence of detection of 6-4 PP and CPD DNA nocytes or fibroblasts) with UVA doses up to 500,000 J/m². This finding significant variation in UDS levels after the irradiation of cells (kerati- patient after exposure to UVB or to UVA irradiation. DISCUSSION To date, only a few studies have analyzed the responses of nontransformed keratinocytes and dermal fibroblasts from normal individuals to UV radiation (25-28). Furthermore, the study of epidermal keratinocytes from XP patients has been limited to the measure of their DNA-repair capacity by UDS after short-term culture and UVC irradiation (29-32). We have undertaken the characterization and comparison of the responses to physiological UV radiation (i.e., UVA and UVB) of dermal fibroblasts and epithelial keratinocytes grown from the skin of normal, XP, and TTD/XP-D individuals. Our main objective was to determine whether specific responses to UV radiation of keratinocytes from XP patients could explain their predisposition to epidermal carcinomas in sun-exposed areas of the skin. In addition, we aimed to clarify the paradoxical question of why XP-D patients are cancer prone, whereas TTD/XP-D patients are not, although the same DNA-repair gene, i.e., XPD, is mutated in both syndromes.

We compared the DNA repair capacities (using the UDS technique), and cell survivals (using the clonal analysis) of fibroblasts and keratinocytes grown in parallel from each XP or TTD/XP-D patient. Our analysis was refined by the study of the clonal type-specific sensitivity of keratinocytes to UV irradiation and by the comparison of the numbers of UVB-induced DNA lesions in keratinocytes and in fibroblasts. All experimental data were analyzed statistically. Our study thus presents the first set of exhaustive and comparative data between primary keratinocytes and fibroblasts from one normal indi-idual, XP (one XP-C and one XP-D) patients, and one TTD/XP-D.

DNA Repair as Measured Using UDS. We failed to detect any significant variation in UDS levels after the irradiation of cells (kerati- nocytes or fibroblasts) with UVA doses up to 500,000 J/m². This finding is in agreement with the absence of detection of 6-4 PP and CPD DNA lesions after UVA irradiation of human skin explants (33). Furthermore, CPDs are hardly detectable by immunocytochemistry after a wide range of UVA irradiation (i.e., 320-400 nm) of skin reconstructed in vitro (6). Because high UVA doses (250 kJ/m²) induce tumors in rodents (34, 35) and are mutagenic both in vivo (36) and in vitro (2), one must admit that specific UVA-specific DNA damages (mostly 7,8-dihydro-8-oxogua-nine), are either not repaired by NER or that their repair by NER remains below the detection limit of UDS. In this respect, it must be considered that repair of CPDs and 6-4 PPs by NER is a non-semiconservative insertion of 29-32 DNA desoxyribonucleotides, whereas a major mode of removal oxidative DNA damages occurs at the level of a single base by a mechanism called base excision repair (37). The UDS technique as performed in our experiments, therefore, is not appropriate to measure UVA-induced oxidative DNA damages.

UVC and UVB radiation induced variable increases of UDS levels, according to the complementation group of both fibroblasts and keratinocytes. Moreover, equivalent doses of both wavelengths (i.e., leading to 50% cell survival) led to comparable numbers of grains in normal cells (either fibroblasts or keratinocytes), suggesting the presence of similar numbers of DNA lesions. UDS levels could be arranged hierarchically in fibroblasts as WT > TTD/XP-D > XP-D > XP-C (in all cases P < 0.001), and in keratinocytes as WT > TTD/XP-D = XP-D > XP-C [P < 0.001 for WT/XP-C; P < 0.01 for (TTD/XP-D)/XP-C; P < 0.01 for XP-C/XP-D; P > 0.05, i.e., not significant, for (TTD/XP-D)/XP-D]. XP-D and TTD/XP-D fibroblasts, but not keratinocytes, could be separated by their capacity to repair UVB-induced DNA lesions, as observed previously in normal fibroblast cultures and in normal human skin explants (33). These experiments thus are not sufficient to explain the predisposition of XP-D, but not TTD/XP-D patients, for epidermal carcinomas. However, due to their respective specific DNA-repair capacities (XP-D cells are deficient in the repair of both 6-4 PP and CPD photolesions, whereas TTD/XP-D are only deficient in the repair of CPDs (16, 20), UDS may underestimate the repair capacity of TTD/XP-D compared with XP-D keratinocytes (see next section). This hypothesis is supported by recent data showing that 25% of CPD induced by a 30 J/m² UVC irradiation are repaired within 3 h after irradiation in normal cells (38). In addition, analysis of DNA repair by the UDS technique is not likely to reflect repair of non-CPD and non-6-4 PP minor
photoproducts induced by UVB irradiation (i.e., formamidopyrimidines and pyrimidine hydrates). Indeed, contribution of these photoproducts to UDS is expected to be negligible because they represent ~1/100 of CPDs and because they are repaired by base excision repair (37).

**Same Doses of Irradiation Lead to Significantly Fewer DNA Lesion Numbers in Keratinocytes than in Fibroblasts.** Following irradiation at the same doses (e.g., 1000 J/m² UVB), the UDS values within a complementation group were systematically inferior in keratinocytes than in fibroblasts irrespective of whether the culture medium contained high (e.g., DMEM) or low (Ham’s F10) thymidine concentrations (data not shown). Concordant observations have been reported previously but remained unexplained (25, 31). Using WT cells, we demonstrated that there were ~50% fewer both 6-4 PP and CPD lesions in keratinocytes than in fibroblasts. This suggests that keratinocytes have developed a natural shielding protection against UV radiation, to which they are exposed much more than dermal fibroblasts. Although differing compactness of chromatin in fibroblasts and keratinocytes could be responsible for this protection, no significant difference in the sizes of nuclei of both cell types could be measured. We suggest that the presence of keratins (39) in keratinocytes, but not in fibroblasts, may perhaps help attenuate UV-induced DNA damages. The larger number of both DNA lesions (6-4 PP and CPDs) found in fibroblasts may explain why XP-D fibroblasts could be separated from TTD/XP-D fibroblasts based on their DNA repair capacity (measured using UDS), whereas homologous keratinocytes could not. This strongly supports our hypothesis that UDS as performed under our conditions underestimates the DNA repair capacity of TTD/XP-D keratinocytes. Additional experiments will compare the number of lesions and their repair kinetics in keratinocytes and fibroblasts with those in normal, XP-C, TTD/XP-D, and XP-D individuals.

**Cell Survival/Clone-forming Ability.** Cell survival of both fibroblasts and keratinocytes following UV irradiation were measured using clonal analysis, a method reflecting the ability of a single cell to restore the integrity of its DNA to the extent necessary for cell division. Whereas this is a quite usual procedure with respect to the measurement of post-UV survival of fibroblasts (40), clonal analysis of keratinocytes from XP and TTD/XP-D has never been used to measure their resistance to UV radiation. This method is, however, much more adequate than any other in the case of keratinocytes. In the epidermis, renewing keratinocytes schematically belong to either the stem or the transient amplifying cell compartment and are mostly located in the basal layer (4, 41). The capacities of these cells to divide can be anticipated in vitro because they can initiate colonies whose morphology and life span reflect quite faithfully their intrinsic growth potentials (see “Results”). In addition, epidermal tumors (BCCs and SCCs) are likely to develop from basal cells with high growth potential that have escaped growth control (e.g., loss of a tumor suppressor gene such as p53, patched). Therefore, clonogenic cells with high growth potential presumably correspond to those cells susceptible of initiating an epidermal tumor (4, 42).

The survival rates of the studied fibroblast strains (each complementation group compared with one another) after UVA irradiation were all significantly different only for doses >250,000 J/m². In general accordance with previous reports (43, 44), this allowed us to draw a survival hierarchy: WT > TTD/XP-D > XP-C > XP-D [P < 0.001 for WT/XP-C and WT/XP-D; P < 0.01 for WT/XP-C and XP-C/TTD/XP-D]. The survival hierarchy after UBV irradiation was WT > TTD/XP-D > XP-C > XP-D [P < 0.001 for WT/XP-C, WT/TTD/XP-D, WT/XP-D, and XP-C/TTD/XP-D]. The survival rates of the WT, TTD/XP-D and XP-D keratinocytes strains studied here did not differ from one another after UVA irradiation below 250,000 J/m². Conversely, our XP-C keratinocytes were clearly more sensitive to UVA radiation than those from any other complementation group studied because their survival decreased at UV doses as low as 100,000 J/m². Other XP-C keratinocytes strains studied in the laboratory exhibited a similar behavior (data not shown). This observation suggests that, under a threshold of 250,000 J/m² UVA, oxidative DNA damages are repaired efficiently in our WT, TTD/XP-D, and XP-D keratinocytes, but not in our XP-C keratinocytes. We have noticed that the expression of metallothionein I, a protein that has been suggested to behave as a scavenger of reactive oxygen species (45), is significantly lower in XP-C than in WT, XP-D, and TTD/XP-D keratinocytes. Low metallothionein levels in XP-C keratinocytes might thus explain their increased sensitivity to UVA radiation. In addition, we are measuring in the keratinocyte strains the expression of enzymes (e.g., haem-oxygenase and superoxide dismutases) that participate in the elimination of reactive oxygen species (46).

**DNA Repair and Cell Survival following UVB Irradiation.** At all doses, the XP-D keratinocyte strain was the cell strain most sensitive to UVB radiation. In contrast, the TTD/XP-D keratinocyte strain was as resistant as the WT keratinocyte strain up to a threshold dose of 450 J/m² UVB. This demonstrates that despite their apparent severe DNA-repair deficiency (which did not allow us to distinguish TTD/XP-D from XP-D keratinocytes), the behavior of the studied TTD/XP-D keratinocytes determined by clonal analysis could be separated clearly from that of the studied XP-D keratinocytes. This observation supports our hypothesis that DNA repair capacities are underestimated when measured by UDS in TTD/XP-D cells and in keratinocytes. The XP-C keratinocyte strain displayed an intermediate survival level to UVB although its DNA repair was the lowest. The fact that UDS does not give much information on the transcription-coupled repair, but rather preferentially reflects the repair of the global genome (38) – i.e., the repair process specifically altered in XP-C cells – may explain the relatively high UVB survival in these cells despite their severe DNA-repair deficiency as estimated by UDS. Our findings thus support the idea that the capacity of a cell to perform transcription-coupled DNA repair efficiently is accompanied by high survival rates after UV radiation. This also explains that TTD/XP-D keratinocytes (whose repair capacity of 6-4 PP is presumably normal with respect to both the global genome and to preferential repair), exhibit significantly higher survival after UVB radiation than XP-D keratinocytes (whose repair deficiency of 6-4 PP and CPD presumably concerns both global and preferential repair pathways; 20).

**Does the UV-induced Transition from Proliferative to Abortive Colony Reflect a Protection against Cancer?** Exposure to both UVA and UVB radiation induced changes in the relative representations of the different colony types (Fig. 4). In general, the numbers of LSP colonies decreased whereas those of abortive colonies (ST) increased. In this respect, it would be interesting to measure the effects of UV radiation on the expression of the proto-oncogene c-myc in the different clonal types whose expression was shown recently to promote the differentiation of epidermal stem cells (47). The behavior of keratinocytes from the XP-C patient studied was distinct from that of other cell strains: ST colonies did not increase significantly after UVB radiation; relative ratios of LSP and W colonies remained constant following UVB radiation, and W colonies increased following UVA radiation (P < 0.02). The relative resistance to UV radiation of cells with high growth capacities (i.e., cells forming LSP and W colonies) may explain the dramatic predisposition of the corresponding patient to develop cancer. Conversely, UV-induced transition from proliferative to abortive colonies might reflect a protection against cancer development. We hypothesized that apoptosis could be selectively responsible for this transition.

**UDS Values following UV Doses Leading to 50% Survival.** In this study we experimentally compared the capacities of keratinocytes and fibroblasts grown in vitro to repair their DNA following UV irradiation. We found that equal doses of UVB induced twice as many lesions...
in WT fibroblasts as in WT keratinocytes when both cell types were grown as monolayers (Fig. 2). Furthermore, our survival curves demonstrated that keratinocytes resist UV radiation better than do fibroblasts. We thus compared the respective DNA repair capacities measured by UDS of normal keratinocytes and fibroblasts after UVB irradiation leading to 50% survival (see Table 1). Under these conditions, UDS values obtained from the cell strains studied were about two to three times lower in fibroblasts than in keratinocytes (see Fig. 2). This indicated that in normal cells the ratio of repair capacity to cell viability is much better in keratinocytes than in fibroblasts (i.e., 15 UDS grains/nuclei versus 5 UDS grains/nuclei for 50% cell survival in keratinocytes and fibroblasts, respectively). In this respect, it would be interesting to measure the repair kinetic of UV-induced DNA lesions present in both keratinocytes and fibroblasts in the skin of normal and DNA-repair-deficient patients following UVA and UVB radiation as performed previously on human skin ex vivo (48) and in vivo (7).

Our study has put forward important clues regarding the predisposition of keratinocytes to develop as tumor cells following solar irradiation. It opens new avenues in the study of keratinocytes protection against solar radiation, including transfer of adequate DNA repair genes in cells from XP patients.

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