Mechanisms underlying DNA damage resistance in a Xiphophorus melanoma cell line

Steven Moredock, Rodney S. Nairn, Dennis A. Johnston, Michelle Byrom, Ginger Heaton, Megan Lowery and David L. Mitchell

The University of Texas M.D. Anderson Cancer Center, Department of Carcinogenesis, Science Park/Research Division, Smithville, TX 78957, USA
1To whom correspondence should be addressed
Email: dmitchell@sprdl1.mdacc.tmc.edu

The Xiphophorus hybrid fish model is an important resource for investigating the genetics and molecular biology of melanoma. Consistent with studies using human melanoma cell lines, the Xiphophorus melanoma cell line PSM, survives the lethal effects of ultraviolet-B radiation (UV-B) radiation much better than a cell line derived from normal fish tissue. In contrast to human melanoma cells, which show enhanced nucleotide excision repair, we do not see any differences in the efficiencies of photoenzymatic or nucleotide excision repair in normal and melanoma cell lines. We do, however, observe a significantly reduced growth rate in the melanoma cell line compared with the normal cell line and considerably less effect of UV-B radiation on DNA synthesis. The data suggest that the UV resistance phenotype of PSM cells is due more to the rate of proliferation and increased ability to replicate on a damaged template rather than enhanced repair of DNA photoproducts as observed in human melanoma cells. The putative increase in lesion bypass by DNA polymerase could result in higher mutation frequencies and enhanced genetic lability in fish melanoma cells.

Introduction

Human malignant melanoma is one of the most rapidly increasing forms of cancer in the West resulting in 47,700 cases and 7,700 deaths expected in America in the year 2000 (American Cancer Society). The incidence of one in 75 Americans (1) is increasing ~3% per year according to the SEER Cancer Statistic Review 1973–1999 (2). The rising prevalence of melanoma is of particular concern as it is less age-dependent than most cancers, metastasizes early in its clinical history and has a poor prognosis if not detected early. Contributing to the poor prognosis of metastatic melanoma is the lack of effective treatment protocols due to the poor response to radiotherapy, chemotherapy and immunotherapy (3). The refractivity of these tumors to nitrosoureas, Vinca alkaloids, cisplatin, dacarbazine, melphalan and ionizing radiation suggests a broad underlying resistance to DNA damage. Studies using UV-irradiated normal and resistant subclones of an established melanoma cell line suggest that the observed refractivity to DNA damaging agents may result from up-regulated nucleotide excision repair (NER) and post-replication repair (PRR) (4).

Inter-specific hybrids among platyfishes and swordtails of the poeciliid fish genus Xiphophorus have been used as genetic tumor models, particularly for malignant melanomas, for more than six decades (5,6). Oncogene and tumor suppressor gene involvement in a variety of spontaneous and chemical carcinogen-induced tumors has been and continues to be extensively studied. Select Xiphophorus inter-species genetic hybrids develop melanoma spontaneously or after acute or chronic exposure to solar UV radiation (7–9). The relevance of this model to the human condition is somewhat problematic considering the broad phylogenetic separation, and yet, it is possible that there is enough genetic and molecular overlap to justify its importance in the study of melanoma.

It is not known if Xiphophorus melanomas show the same resistance to therapy as human melanomas. However, a normal and melanoma fish cell line are available that can be used to address this question and determine if such differences in survival exist and whether NER or PRR plays a part in this resistance. Little work has been done characterizing the basic biological response of these cell lines to DNA damaging agents. The experiments presented in the current study were designed to parallel those of Hatton and co-workers (4) and determine if fish melanoma cells show the same refractivity to DNA damaging agents and enhanced DNA repair observed in human melanoma cells. In contrast to the earlier experiments, which used ultraviolet-C radiation (UV-C) as the damaging agent, we used ultraviolet-B radiation (UV-B) to characterize several responses of A2 and PSM cells, including growth and survival, photoenzymatic repair (PER) and NER, and DNA synthesis.

Materials and methods

Cells and cell culture

Late embryonic tissue from Xiphophorus siphidium was used to establish a normal cell line designated A2 (10) and tissue from an amelanotic melanoma from an F1 hybrid of Xiphophorus maculatus × albino Xiphophorus helleri fish was used to derive a melanoma cell culture designated PSM (11). A2 and PSM cells were obtained from M. Shartl (Wurzburg University). A2 cells were maintained in α-Modified Dulbecco’s Minimal Essential Media (α-MEM) and PSM cells were grown in F12 Nutritional Media (Gibco BRL), both supplemented with 10% fetal bovine serum (Irvine Scientific) and 100 IU penicillin and 100 μg/ml streptomycin (Gibco BRL). A2 and PSM cells were generally re-plated at 1:2 or 1:3 density. Cells were kept in a humidified atmosphere with 5% CO2 at 24°C. Morphologically the A2 cells appeared cuboidal and of epithelial origin whereas the PSM melanoma cells were dendritic and resembled melanocytes.

Light sources and UV irradiation

UV-B radiation was administered inside an irradiation chamber containing four unfiltered Westinghouse FS20 sunlamps emitting predominantly 280–360 nm light. UV-B fluorescence was quantified with a UV-B-1 probe (International Light) coupled to a Model IL 1400A radiometer/photometer (International Light). Fish cells were irradiated in Hank’s buffered saline solution.
solution (HBSS) in a covered culture dish (Owens-Corning) that excluded wavelengths <290 nm. Photoreactivating light (PRL) was supplied by fluorescent ‘Cool White’ lamps (General Electric) filtered through Mylar 500D film (DuPont) to exclude wavelengths below 320 nm. For PER experiments, cells remained in HBSS throughout the white light exposure. Yellow lights were used at all times to minimize any stray white light effects on photoproduct frequency.

**Growth rate experiments**

Cells were harvested from culture flasks with 5% trypsin and vigorously mixed at 4°C to assure single cell suspension. After quantification with a Coulter Counter (Coulter Electronics, Hialeah, FL) cells were diluted with cold media to the appropriate plating density. For control growth experiments, cells were plated at 0.6–1.0 × 10^6 cells/60 mm plate; for UV-B growth experiments, cells were plated at 0.6–1.0 × 10^5 cells/60 mm plate. Cells were counted at 24 h intervals. For higher density samples, cells required aspiration through a sterile 18 g needle. Three aliquots were drawn from each of two replicate plates and counted four times.

**Survival experiments**

Approximately 10,000 cells were plated in 60 mm culture plates with grid lines at 2 mm intervals (Owens-Corning) and incubated for 24 h prior to irradiation. Groups of six plates were exposed to increasing doses of UV-B radiation, treated with PRL or not, and returned to the incubator until small colonies (> 8 cells) could be detected in the unirradiated samples. Media was replaced every 5 days for α-MEM and every 5 days for F12. For each plate a minimum of 75 grids was scored with adjacent rows of grids counted back and forth across the middle of the plate. Grids near the perimeter of the plate were not counted. The number of colonies was normalized to the unirradiated control.

**DNA damage induction and repair**

Groups of four A2 or six PSM 100 mm culture plates were used for each sample (i.e. UV-B dose or repair time). Prior to irradiation, the edges of the plate were swabbed with a cotton-tipped applicator to remove cells that may be shaded by the rim of the plate. Cells were irradiated at 90% confluency in HBSS and either harvested immediately for photoproduct induction experiments or returned to the incubator in fresh medium for designated repair times. PER cells were immediately placed under white light after UV-B exposure for a designated period (usually 1 h) after which they were either harvested or returned to the fresh medium in time. At the time that harvest the cells were lysed over night in 10 mM Tris, pH 7.8; 1 mM EDTA; 0.3% SDS; 0.3 mg/ml Proteinase K (Boehringer-Mannheim), extracted with an equal volume of chloroform:isoamyl alcohol and the DNA precipitated overnight in ethanol at −20°C. Prior to assay the sample DNA was denatured at 100°C for 10 min and quantified using the Oligene ssDNA fluorescence assay (Molecular Probes) and an FL-600 Microplate Fluorescence Reader (Bio-Tek Instruments, Winoski, VT).

**Radioimmunoassay of DNA photoproducts**

Antisera were raised against DNA that was either irradiated with 100 kJ/m² UVC (254 nm) radiation for pyrimidine(6-4)pyrimidone dimer [(6-4)PDs] or dissolved in 10% acetic acid and irradiated with UV-B radiation under conditions that have been shown to produce cyclobutane pyrimidine dimers (CPDs) exclusively. For the RIA 2.5-5 μg of heat-denatured sample DNA, was incubated with 5–10 pg of poly(dA):poly(dT) (labeled to >5 × 10^6 c.p.m./μg by nick translation with 32P-dTPP) in a total volume of 1 ml 10 mM Tris, pH 7.8, 150 mM NaCl, 1 mM EDTA and 0.15% gelatin (Sigma). Antiserum was added at a dilution that yielded 30–60% binding to labeled ligand and after incubation overnight at 4°C the immune complex was precipitated with goat anti-rabbit immunoglobulin (Calbiochem) and carrier serum from non-immunized rabbits (UTMDACC, Science Park/Veterinary Division, Bastrop, TX). After centrifugation, the pellet was dissolved in tissue solubilizer (NCS, Amersham), mixed with ScintiSafe (Fisher) containing 0.1% glacial acetic acid, and the 32P quantified by liquid scintillation spectrometry. Under these conditions, antibody binding to an unlabeled competitor inhibits antibody binding to the radiolabeled ligand. Sample incubation is extrapolated through a standard (dose–response) curve to determine the number of photoproducts in 10^7 bases [i.e. CPDs or (6-4)PDs/mM]. For standard we used double-stranded salmon testes DNA (Sigma) irradiated with increasing doses of UV-C radiation and heat-denatured, aliquoted and kept frozen at −20°C. Rates of photoproduct induction were previously quantified using non-immunological enzymatic and biochemical techniques and determined to be 8.1 CPDs and 1.56 (6-4)PDs/mM, respectively. These details, as well as those concerning the specificities of the RIAs, are described in Mitchell (12,13).

**DNA synthesis**

Approximately 300–400,000 A2 or PSM cells were plated in 60 mm plates in α-MEM or F12 media supplemented with 0.25 μCi/ml [3H]thymidine (Tdr), allowed to grow for 48 h, and returned to non-radioactive media for 24 h prior to the experiment. Immediately prior to irradiation, plates were washed with Solution A (137 mM NaCl, 5.4 mM KCl, 5.6 mM d-glucose and 4.2 mM NaHCO3, pH 7.5) and exposed in triplicate in Solution A to 0.1, 0.25, 0.5, 1, 2, 4 and 8 kJ/m² filtered UV-B. After exposure Solution A was removed and replaced with α-MEM or F12 media. At 2 h post-irradiation 2 ml of either α-MEM supplemented with 1 μCi/ml [3H]Tdr or F12 media supplemented with 5 μCi/ml [3H]Tdr was added for an additional 30 min pulse. Cultures were then washed 2× with ice cold Solution A and fixed with two 5 min treatments with ice cold 10% trichloroacetic acid (TCA). After TCA cells were lysed with 2 ml 0.3 N NaOH, 1% SDS for 15 min at 23°C and decanted to a scintillation vial. Radioactivity was measured in a liquid scintillation counter (Packard Instruments) programmed to convert c.p.m. to d.p.m. and quantify the ratio of the [3H]/[14C].

**Statistical analyses**

The growth and repair data, sample sets were compared with ANOVA using the SPSS statistics program (1995). Regression statistics were obtained with SPSS and further analyzed using the AOCslope program, which statistically compares slopes and elevations (γ-intercepts) with the Tukey HSD Comparison (D.Johnston, MDACC, Houston, TX). In addition, the experimental repair data were fitted to the exponential decay function y = r + ae^−bt where b is the amplitude (amount of damaged induced by 6 kJ/m² independent of existing residual damage), y is the inverse of the repair half-life and r is the amount of residual (unrepairable) damage (SigmaPlot v. 8.0, SPSS).

**Results**

**UV-B effects on cell division**

For baseline growth rate determinations A2 and PSM cells were plated at sub-confluent densities and cell numbers were measured at 24 h intervals after plating (Figure 1). The average cell counts for A2 on days 1–13 and for PSM on days 1–25 were fitted to an exponential model using regression analysis and doubling times of 44 (R² = 0.974) and 68 h (R² = 0.969) were calculated for the A2 and PSM cells, respectively. Observed monolayer densities for A2 and PSM cells were

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Fig. 1. Growth kinetics of A2 and PSM cells. Approximately 10^5 cells were plated onto 60 mm plates and cell densities determined at 24 h intervals. Shown are the mean and standard deviation. R² for the sigmoidal plots were 0.9867 and 0.9885 for A2 and PSM, respectively. A2, (○); PSM, (●).
~2000 and ~1000 cells/mm², respectively. The PSM cells reached saturation at ~3.2 \times 10^6 \text{ cells/plate} at which point masses of loosely attached cells were observed in dome-shaped colonies. Cells that dislodged from these masses were plated and found to be viable. In contrast to the PSM cells, the A2 cells did not saturate but reached a density of ~5.5 \times 10^6 \text{ cells/plate} at which point most of the cells could be easily (mechanically) dislodged from the plate. Whereas very few of the unattached cells were viable, those that remained attached were sufficient to eventually re-populate the plate. Hence, neither A2 nor PSM showed any contact inhibition.

The effects of UV-B exposure on population growth kinetics in A2 and PSM was determined in the presence and absence of PRL (Figure 2A and B). PER of CPDs and (6±4)PDs is a common and well-utilized repair mechanism in fish in general (14) and in \textit{Xiphophorus} fish in particular (15,16). For these experiments a UV-B dose was selected that yielded 37% survival for each cell line (i.e. the D37 dose). For the A2 cells, a significant inhibition in cell division was observed up to 4 days after irradiation at which time the cultures entered an exponential growth phase (Figure 2A). The initial delay in cell growth was more protracted in the cells not exposed to PRL. Comparison of these curves using ANOVA indicated that, whereas the irradiated cultures grew significantly different compared with the control (\( P < 0.001 \)), there was no significant difference between the two irradiated treatment groups (±PRL) (\( P = 0.425 \)). Slopes of the different treatment groups were calculated for days 4–8 and are shown in Table I. It is evident that exponential growth in the UV-B irradiated A2 cells is significantly greater (3-fold) than in the unirradiated cells. In contrast to A2, the growth rate of the PSM cells was significantly reduced after a dose of UV-B radiation that showed equivalent levels of killing in both cell lines (Figure 2B). Statistical analysis of the slopes using AOCslope showed a significant difference between the treated and untreated groups (\( P < 0.001 \)) but no significant difference in the slopes of the two treated groups (±PRL) (\( P = 0.089 \)) (Table I). Growth rates in the treated cells were 20–25% of the unirradiated control. Statistical analysis of the curves did, however, show significant differences between the y-intercepts (\( P < 0.001 \)) indicating a possible faster recovery for the PRL-exposed group.

### Survival

Survival for A2 and PSM cells exposed to increasing doses of UV-B (±PRL) is shown in Figure 3 (no PRL survival curves were generated). As a result of slow growth and deciduous properties of the fish cell lines used, we found it necessary to reduce the minimum size of colonies from 50 (in mammalian cultured cells) to ~8 cells (i.e. at least three doublings). Beyond this time colony formation depended more on cell detachment and migration rather than UV exposure. It is evident that PSM cells are considerably more able to survive the killing effects of UV-B.

#### Table I. Growth rates for A2 and PSM cells after equitoxic doses of UV-B radiation

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Control</th>
<th>UVB-PRL</th>
<th>% control</th>
<th>VB</th>
<th>PRL</th>
<th>% control</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>0.73 (0.82)</td>
<td>2.13 (0.38)</td>
<td>292</td>
<td>2.35 (0.25)</td>
<td>322</td>
<td></td>
</tr>
<tr>
<td>PSM</td>
<td>0.38 (0.18)</td>
<td>0.08 (0.01)</td>
<td>21</td>
<td>0.11 (0.01)</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as the mean and standard error (\( \times 10^6 \text{ cells/day} \)).
of UV-B than the A2 cells ($P = 0.002$). Both cell lines show 10–20% cell killing at 10 J/m² and nearly 50% killing at 50 J/m², doses which would be sublethal in mammalian fibroblast cultures (17). Above 50 J/m² the curves diverge with the A2 and PSM cells showing 0 and 40% survival at 125 J/m², respectively. D37 doses were calculated from linear regression curves for all of the data and found to be 71 J/m² for the A2 and 127 J/m² for the PSM cells. Hence, the melanoma cell line is about twice as resistant as the epithelial cell line to the killing effects of UV-B.

Photoproduct induction

Dose–response curves were generated for the induction of CPDs and (6–4)PDs in A2 and PSM cells (Figure 4) and were found to be linear with dose (Table II). Regression

Table II. Induction rate parameters for CPDs and (6–4)PDs in A2 and PSM cells

<table>
<thead>
<tr>
<th>Photoproduct</th>
<th>Cell line</th>
<th>$R^2$ a</th>
<th>Slope b</th>
<th>D37 Load c</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPD</td>
<td>A2</td>
<td>0.959</td>
<td>0.466</td>
<td>33</td>
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<tr>
<td></td>
<td>PSM</td>
<td>0.922</td>
<td>0.611</td>
<td>78</td>
</tr>
<tr>
<td>(6–4)PD</td>
<td>A2</td>
<td>0.948</td>
<td>0.057</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>PSM</td>
<td>0.859</td>
<td>0.103</td>
<td>13</td>
</tr>
</tbody>
</table>

aCorrelation coefficient (SPSS).
b(lesions/mb)/(J/m²).
cLesions/mb.

Fig. 3. Colony-forming ability of A2 and PSM cells in response to UV-B radiation. Approximately $10^4$ cells were plated onto 60 mm plates and exposed to increasing doses of UV-B radiation. Mean and standard deviation are shown. A2, (○); PSM, (●).

Fig. 4. Photoproduct induction in A2 and PSM cells. Cells were irradiated in 60 mm plates at 90% confluence with increasing doses of UV-B radiation and photoproduct frequencies determined by RIA. Mean and standard deviation are shown for CPDs in (A) and (6–4)PDs in (B). A2, (○); PSM, (●).
statistics indicated that the rate of CPD induction was higher, but not significantly higher in PSM compared with A2 cells. In contrast, the rate of (6±4)PD induction was significantly higher in A2 cells compared with PSM. This result is somewhat problematic since the relative induction of CPDs and (6±4)PDs should not be different in the two cell lines. However, from the repair data shown in Figures 6A and 8A the amount of damage initially induced is very comparable for the two cell lines when adjusted for the difference in D$_{37}$ dose. We conclude that the CPD and (6±4)PD induction rates in A2 and PSM cells are the same. Using a photoproduct induction rate of 0.54 CPDs/mb/Jm$^2$ (from Table II) (the average of the A2 and PSM induction rates in Table II) and D$_{37}$ values determined from the survival curves, we calculated that PSM cells could sustain about twice as much DNA damage as the A2 cells.

**Photoenzymatic repair**

Two DNA repair pathways for removing CPDs and (6±4)PDs were examined in the two different *Xiphophorus* cell lines. In Figure 5, PER of CPDs induced by the D$_{37}$ dose for each cell line is shown and is seen to be very efficient in both A2 and PSM cells. Initial slopes of A2 and PSM PER were calculated from the exponential decay curves and shown to be nearly identical ($t_{1/2}$ = 0.45 and 0.47 h) (Figure 5A). These rates are comparable with those observed in vivo in *X.variatus* and *X.signum* (15,16). When the data are normalized to the 0 h control, we see that although a greater proportion of the damage is removed by the A2 relative to the PSM cells, statistical analysis showed no significant difference between the slopes of the curves (Figure 5B; Table III). The data indicate that PER efficiency in A2 and PSM cells is comparable.

PER of (6±4)PDs also occurs in *Xiphophorus* (15,16). PER of (6±4)PDs is considerably less efficient than CPDs with estimated half-lives ($t_{1/2}$) of ~6 and ~3 h for A2 and PSM cells calculated from the exponential decay curves. The high variability observed when analyzing the very low levels of damage necessitated by the D$_{37}$ dose precluded any definitive statistical analysis.

**Nucleotide excision repair**

For NER studies care was taken to avoid any stray white light 24 h prior to and during the experiment when cells were either manipulated under yellow lights or kept in a dark CO$_2$ incubator. From the data shown in Figure 7A it is evident that CPD removal by NER is considerably slower than PER and appears to be biphasic. Like PER, these kinetics are similar to those

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Data</th>
<th>Slopes (photoproduct or %/mb/h)</th>
<th>AOCslope</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>SE</td>
</tr>
<tr>
<td>CPD</td>
<td>Actual</td>
<td>−33.5</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Normalized</td>
<td>−71.4</td>
<td>15.9</td>
</tr>
<tr>
<td>(6±4)PD</td>
<td>Actual</td>
<td>−1</td>
<td>−1</td>
</tr>
<tr>
<td></td>
<td>Normalized</td>
<td>−4.5</td>
<td>−20</td>
</tr>
</tbody>
</table>

*aLesions/mb.

b% of unirradiated control.

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**Fig. 5.** Photoenzymatic repair of cyclobutane dimers in A2 and PSM cells. Cells were irradiated with respective D$_{37}$ doses and exposed to 1 h PRL. Cells were harvested at appropriate repair intervals and CPD frequencies measured by RIA (A). In (B), the data are normalized to 100% at $t_0$. Mean and standard deviation are shown for A2, (○) and PSM, (●).
Fig. 6. Photoenzymatic repair of (6-4) photoproducts in A2 and PSM cells. Cells were treated and harvested as in Figure 5. (6-4)PD frequencies were measured by RIA (A) and are shown normalized to 100% at t₀ in (B). Mean and standard deviation are shown for A2, (*) and PSM, (●).

Fig. 7. Nucleotide excision repair of cyclobutane dimers in A2 and PSM cells. Cells were irradiated with respective D₁₀ doses and harvested at appropriate repair intervals for CPD analysis using RIA (A). In (B), the data are normalized to 100% at t₀. Mean and standard deviation are shown for A2, (*) and PSM, (●).
observed in vivo (15,16). ANOVA of the normalized data shown in Figure 7B showed no statistical difference between the A2 and PSM cell lines in the rates of NER ($P = 0.13$). In addition, regression analysis of the slopes (Table IV) indicated A2 and PSM cells repaired CPDs at statistically indistinguishable rates ($P = 0.36$). Excision repair of the (6–4)PD was significantly faster than the CPD (Figure 8A) but gave similar results when the two cell lines were compared. ANOVA of the normalized data shown in Figure 8B showed no statistical difference between the A2 and PSM cell lines in the rates of (6–4)PD repair ($P = 0.88$). In addition, regression analysis of the slopes (Table IV) indicated A2 and PSM cells repaired (6–4)PDs at statistically comparable rates ($P = 0.69$). We conclude from the data that A2 and PSM cells have very similar NER capacity.

### Table IV. Regression statistics for CPD and (6–4)PD NER in A2 and PSM cells

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Data</th>
<th>Slopes (photoproduct or %/mb/h)</th>
<th>AOCslope</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>SE</td>
</tr>
<tr>
<td>CPD</td>
<td>Actual$^b$</td>
<td>−1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Normalized$^b$</td>
<td>−3</td>
<td>0.4</td>
</tr>
<tr>
<td>(6–4)PD</td>
<td>Actual</td>
<td>−0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Normalized</td>
<td>−10</td>
<td>0.1</td>
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*aLesions/mb.

**UV-B effects on DNA synthesis**

The effects of UV-B radiation on semi-conservative DNA synthesis in A2 and PSM cells are shown in Figure 9. Cells were pre-labeled with [14C]TdR, exposed to increasing doses of UV-B and pulsed at 2 h for 30 min with [3H]TdR to quantify nascent DNA synthesis. The ratio of nascent to pre-labeled DNA ([H]:[14C]) was determined from d.p.m. and the unirradiated control was used to normalize the percentage of TdR uptake for each UV-B dose. The contribution of unscheduled DNA (repair) synthesis (UDS) would be negligible even at the highest dose used (i.e. 4 kJ/m²). At 4 kJ/m² ~600 lesions/mb would be induced of which a maximum of ~30 (5%) could be repaired during the 30 min pulse. With an average patch size of 30 bases (18) the maximum incorporation would be ~900 bases/mb or ~0.1% of the total DNA at the highest UV-B dose used (4 kJ/m²) and only 23 bases/mb or ~0.003% of the total DNA at the lowest UV-B dose used (0.1 kJ/m²). In an asynchronous population with a doubling time of 44 or 68 h and further protracted by UV-B exposure, the amount of DNA that could be replicated in 30 min is < 1.0% (0.5/48 h) or 10-fold higher than any putative UDS from the highest dose used. With this in mind, it is evident that DNA synthesis is inhibited in a dose-dependent manner in both A2 and PSM cells with the A2 cells considerably more sensitive to DNA synthesis inhibition than PSM. Indeed, at the lowest dose tested we observed 60–70% inhibition of [3H]TdR incorporation in A2 compared with ~10% in PSM. The difference in rates of DNA synthesis in the two cell lines significantly exceeds that expected from the difference in growth rates.

![Fig. 8. Nucleotide excision repair of (6–4) photoproducts in A2 and PSM cells. Cells were treated and harvested as in Figure 5. (6–4)PD frequencies were measured by RIA (A) and are shown normalized to 100% at t₀ in (B). Mean and standard deviation are shown for A2, (○) and PSM, (●).](https://academic.oup.com/carcin/article-abstract/24/12/1967/2390366)
Discussion

In a previous study we examined the molecular basis for radioresistance in human melanomas by comparing NER and PRR in normal and melanoma cells displaying different UV sensitivities (4). The current study was designed to examine these same endpoints in normal and melanoma cell lines from *Xiphophorus* and further characterize the fish melanoma model. Several differences between the current study and the work done on human cells should be noted. First, whereas UV-C light sources were used for the human studies we used UV-B lamps in the present work. We do not consider this of major importance since the same photoproducts are induced by both spectra, albeit at significantly different rates (i.e. UV-C = 100°UV-B). More significant is the obvious difference that we are comparing cells from two widely separated species. The D$_{37}$ dose for UV-B survival in the A2 cells is 70 J/m$^2$ compared with 700 J/m$^2$ in human fibroblasts (e.g. HeLa, GM637), indicating that this particular fish cell line is ∼10-fold more sensitive to UV-B than human cells.

Similar to human cells, significant differences were observed in the UV stress response of a normal and melanoma *Xiphophorus* cell line. Two caveats should be considered prior to discussion of the results. First, the A2 and PSM cells lines were derived from different species, A2 from *X.xiphidium* and PSM from an F$_1$ hybrid of *X.maculatus* × albino *X.helleri*. Because these were the only extant *Xiphophorus* cell lines (excluding subclones), we were greatly limited in our comparison. Indeed, significant differences in NER are observed in *vivo* between different *Xiphophorus* species and hybrids (19). Secondly, A2 are epitheloid cells and PSM are transformed melanocytes. Differences in cell type may account for some of the differences in growth rate and stress response, however, human epithelial cells and melanocytes show similar responses to UV with comparable NER (20). Our data support the contention that differences in the UV responses between the A2 and PSM cells reflect more the consequences of malignant transformation rather than differences in species or cell-type.

Significant differences in the growth kinetics of the A2 and PSM cells were observed. The A2 cells grew ∼50% faster than the PSM cells under the same culture conditions consistent with rates observed in human cell lines in which melanoma cells grow considerably faster than melanocytes. Because of their size and morphology the monolayer density (confluence) of the epitheloid cells was about twice that of the melanoma cells. Although neither cell line displayed contact inhibition the PSM cells did show higher tolerance for growth saturation; at high saturation (i.e. 3–5 × 10$^6$ cells/60 mm plate) the PSM cells remained viable whereas most of the A2 cells died. This behavior is typical of human melanoma cell lines. The difference in growth rates could indeed contribute to the differences observed in UV survival with PSM cells allowed considerably more time to repair the DNA damage prior to replication.

For the studies on the growth response to UV-B and DNA repair we exposed cells to a dose that yielded equivalent cell killing determined as the D$_{37}$ dose from the survival curve. Using this approach any effects resulting from cell death (e.g. photoproduct dilution) could be obviated. The response in growth kinetics to UV-B irradiation was dramatically different in the two cell lines. Little effect on A2 cell growth was observed until day 5 after the exposure at which time the growth rate of the irradiated cells increased to >300% of the unirradiated control. In contrast, UV exposure had a significant effect on PSM growth, reducing both the slope of the growth curve and the level of saturation/survival. These results are not surprising as the mitogenic response of epitheloid cells and lack of this response in melanocytes is well-documented (21,22).

The overriding impact of mitogenesis on A2 growth does, however, compromise our comparison between the two cell lines since any definitive effects on A2 proliferation are masked by the up-regulation of cell proliferation evident at 5 days post-irradiation. Although comparable UV inhibition is observed in both cell lines prior to 5 days the differences between the treated and control cells are not statistically significant for the A2 cells. At first, these results do not appear consistent with the survival data which show that PSM cells can tolerate about twice as much DNA damage as the A2 cells. However, by the time enhanced cell growth is initiated in the A2 cells (i.e. at 5 days) most of the DNA damage is repaired and survival has been determined. The effects of UV on cell growth are much clearer in the PSM cells where the kinetics are not confounded by a mitogenic response. Here the growth rate is significantly depressed with cells apparently saturating at a level consistent with the 37% expected survival.

As mentioned, the PSM cells are about twice as resistant at the doses ~50 J/m$^2$ as the A2 cells to the lethal effects of UV-B measured as colony-forming ability. To better understand the molecular mechanisms that underlie this difference, we compared the NER efficiencies of the two cell lines and found no significant differences. The data differ from those found for human melanoma cells, which display enhanced NER.
compared with more sensitive cell lines (4). We did, however, observe significant differences in the effects of UV-B on DNA synthesis in the two cell lines with PSM cells showing considerably less effect of UV-B on DNA synthesis compared with A2. The data correlate well with survival and suggest that, as observed in human melanoma cells, enhanced PRR may contribute to the greater recovery of PSM cells after UV exposure compared to A2. Since the DNA damage induction and repair rates are comparable in A2 and PSM cells the replication machinery encounters comparable levels of DNA damage, suggesting that the DNA polymerases utilized by the PSM cells in response to UV have a considerably higher read-through capacity. This property of PSM cells would serve to increase the likelihood of error-prone DNA replication and further increase genetic instability during tumorigenesis.

Our results, limited by the paucity of available fish cell lines, provide a meaningful comparison of fish and human melanoma cells and add insight into mechanisms underlying survival in these cells. We find that, like the human condition, Xiphophorus melanoma cells are resistant to DNA damage. Unlike the human cells, in which enhanced NER and PRR were both implicated in melanoma resistance, growth rates coupled with a greater capacity to replicate on a damaged DNA template appear to determine UV-resistance in the fish melanoma cells. The ability of the fish melanoma cells to replicate more damaged DNA than normal increases the likelihood of continued NER and enhanced survival at the expense of increased mutagenesis. Comparative gene expression analyses focusing on various DNA polymerase genes as well as other genes involved in cell proliferation and the UV stress response would be an appropriate avenue for future study.

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References


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