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ORIGINAL ARTICLE

Genomic instability in radial growth phase melanoma cell lines after ultraviolet irradiation

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Background/Aims: Although ultraviolet (UV) irradiation, apoptosis, and genomic instability are all potentially involved in the pathogenesis of melanoma, in vitro studies investigating these changes in the radial growth phase of this neoplasm are still lacking; therefore, this study was designed to investigate these changes.

Method: An in vitro system consisting of three radial growth phase Wistar melanoma cell lines (WM35, WM3211, and WM1650) was established. Cells were UV irradiated (10 mJ/cm^2 for UVB and 6 J/cm^2 for UVA), harvested after UV exposure, and evaluated for viability and apoptosis using Trypan blue and terminal deoxynucleotidyl transferase mediated dUTP digoxigenin nick end labelling assays, respectively. Polymerase chain reaction based microsatellite assays were used to examine the cell lines for the presence of microsatellite instability (MSI) using 21 markers at the 1p, 2p, 3p, 4q, 9p, and 17p regions.

Results: Exposure to UV initiated progressive cell death associated with pronounced apoptosis, with UVA having a greater effect than UVB. MSI was found in UVB (WM35 and WM3211) and UVA (WM35) irradiated cell lines at 1p, 9p, and 17p, but not in non-irradiated cells. The prevalence of MSI was higher after UVB irradiation (14%) than UVA irradiation (4.7%), and was most frequently found at D1S233.

Conclusions: The ability of erythemogenic UV irradiation to induce both apoptosis and MSI in radial growth phase melanoma cells is suggestive of its role in melanoma pathogenesis. This instability may reflect a hypermutability state, oxidative stress induced DNA damage, replication infidelity, or a combination of these factors.

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Ultraviolet irradiation (UVR) is an important aetiological factor in the development of cutaneous malignant melanomas (CMMs), acting both as an initiator and a promoter in the course of multistep melanoma tumorigenesis.[1] In this regard, exposure to ultraviolet B (UVB) leads to the formation of cyclobutane pyrimidine dimers and pyrimidine (4–6) pyridone photoproducts. The incorrect repair of these products generates mutations and increases genetic instability.[2,3] Exposure to UVB also leads to transcriptional activation of several oncogenes,[4] and the generation of photoxidative stress, which inhibits the anti-oxidative defence mechanisms.[5] In contrast, UVA genotoxicity is induced by the generation of reactive oxygen radicals,[6] which can cause DNA damage that is potentially tumorigenic.[7]

> "Several in vivo studies have revealed the involvement of ultraviolet irradiation, apoptosis, and microsatellite instability in the advanced stages of cutaneous malignant melanoma"

Microsatellites are repetitive DNA sequences of 1–6 base pairs scattered primarily in the non-coding regions of DNA. Neither the factors that restrict them to these regions nor their exact biological roles are clear.[8] Microsatellite instability (MSI) is a variation in microsatellite pattern length between tumorous and matching non-tumorous tissues.[8] MSI can be separated into high (MSI-H; > 30% of tested markers), and low (MSI-L; < 30% of tested markers) instability patterns. MSI-H has been described in families with hereditary non-polyposis colorectal cancer and is associated with mutations in mismatch repair genes. Although MSI-L has also been reported in several tumours,[8] including benign naevi, melanocytic dysplastic naevi,[8] primary CMM,[8,9] and metastatic CMM,[8] the underlying mechanisms of this instability pattern are still unknown.

Several in vivo studies have revealed the involvement of UVR,[10–12] apoptosis, and MSI[8,9] in the advanced stages of CMM. In these tumours, several molecular pathways mediate the activation of a variety of prosurvival and proapoptotic proteins that seem to play a role in melanoma tumorigenesis.[13] However, in vitro studies examining these changes in the early stages of CMM are scarce. Therefore, we used an in vitro system consisting of control (non-irradiated) and irradiated Wistar melanoma (WM) cell lines (non-tumorigenic, radial growth phase cells) to explore cell viability, apoptosis, and genomic instability after UVR. We divided the cell lines into three groups: UVA irradiated, UVB irradiated, and non-irradiated (control) groups. We carried out three sets of experiments to investigate the following questions: (1) what is the effect of a single exposure to UVA or UVB on cell viability? (2) What is the effect of a single exposure to UVA or UVB on apoptosis? (3) What are the genetic alterations in microsatellites associated with a single UVR dose in radial growth phase melanomas?

MATERIALS AND METHODS

Cell culture

Three human WM cell lines (a generous gift from Dr M Herlyn, Wistar Institute, Philadelphia, USA), derived from (non-tumorigenic) radial growth phase CMMs, were established as described by Dr Herlyn (fig 1). Two cell lines (WM35 and WM3211) were maintained in DMEM medium supplemented with 10% fetal calf serum and insulin (5 mg/ml), and the third one, WM1650, was maintained in

Abbreviations: CMM, cutaneous malignant melanoma; LOH, loss of heterozygosity; MED, minimal erythema dose; MSI, microsatellite instability; MSI-H/L, high/low microsatellite instability; PCR, polymerase chain reaction; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP digoxigenin nick end labelling; UVA/B, ultraviolet A/B; UVR, ultraviolet irradiation; WM, Wistar melanoma

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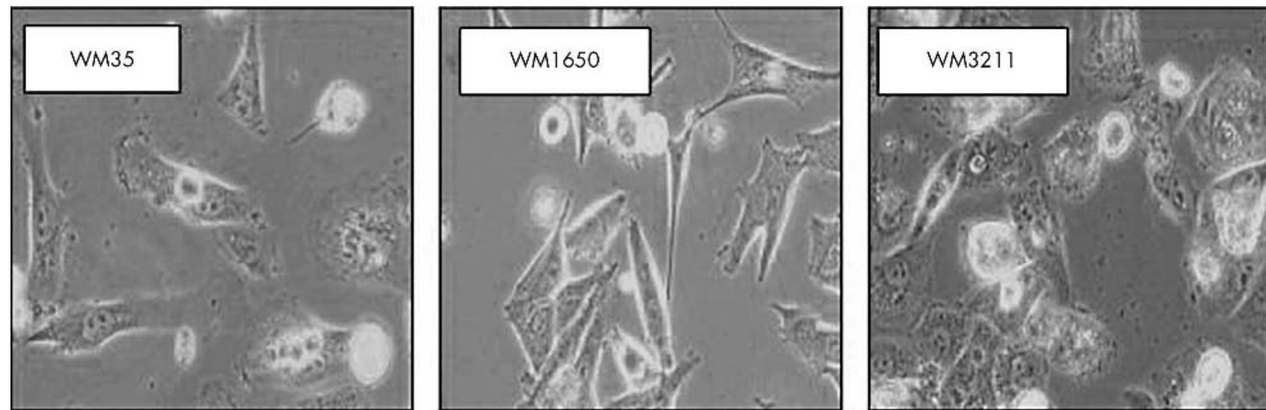

Figure 1 Three human Wistar melanoma cell lines, derived from (non-tumorigenic) radial growth phase melanomas, were established as described by the supplier. Two cell lines (WM35 and WM3211) were maintained in DMEM medium supplemented with 10% fetal calf serum and insulin (5 mg/ml), and the third one, WM1650, was maintained in RPMI medium supplemented with 10% fetal calf serum and 10 ng/ml TPA (12-*O*-tetradecanoylphorbol 13-acetate).

RPMI medium supplemented with 10% fetal calf serum and 10 ng/ml TPA (12-*O*-tetradecanoylphorbol 13-acetate).

The rationale for selecting specific UVR dosages

A dose of 10 mJ/cm^2 UVB was used according to the following rationale: (1) in vivo, doses of UVB in the range of 30 to 150 mJ/cm^2 (~ 1–5 minimal erythema doses; MED) are normally encountered and (2) erythema induced by solar UVB results from the penetration into the dermis of approximately 10% of the total UVB dosage. Therefore, in vitro UVB doses ranging from 3 to 15 mJ/cm^2 (or 1–5 MED dermal equivalents) are equivalent to the amount of irradiation reaching melanocytes at the basal layer that is needed to cause sunburn (~ 1 MED).[14 15] In contrast, for UVA, the transmission through the epidermis is ~ 19% (at wavelength 365 nm), and its MED is 15–20 J/cm^2. The 6 J/cm^2 dosage used for UVA is the minimum dose that can induce oxidative stress and compromise the antioxidant enzyme system. Of note, these last two effects are responsible for the potential carcinogenic capability of UVA.[16–18]

UV irradiation

The cells were maintained in tissue culture flasks (25 cm^2) until they attained confluence. The cells were then trypsinised, suspended in medium, and seeded into several 100 mm^2 petri dishes (1 × 10^5 cells/ml) in phosphate buffer saline. For UVB irradiation, the cells were irradiated under a bank of six FS-40 UVB lamps (Philips, Bloomfield, New Jersey, USA) from which UVB and UVC wavelengths, not normally present in natural solar radiation, were filtered out using Kodacel cellulose film (Eastman-Kodak, Rochester, New York, USA). The light contained radiation in the wavelength of the UVB range (290–320 nm). The total dose of irradiation (10 mJ/cm^2 for UVB) was calculated using a radiometer (IL-700) with UVB detectors (SED240; International Light Inc, Newburyport, Massachusetts, USA). For UVA irradiation, the cells were irradiated under a bank of two Q-Panel UVA (UVA-340 lamp, ASTMG-53) fluorescent lamps (Q-Panel, Cleveland, Ohio, USA). Most of the radiation from this lamp is in the UVA region (320–400 nm), with a small amount in the UVB spectrum. The total dose of irradiation (6 J/cm^2 for UVA) was calculated using a radiometer (IL-700) with UVA detectors (SED033; International Light Inc). Immediately after irradiation, the phosphate buffered saline was removed, and the cells were overlaid with fresh medium and returned to the incubator. Controls (non-irradiated cells) were handled similarly, but were not exposed to UV irradiation. The cells were evaluated for viability, apoptosis, and the presence of genetic changes (including MSI and loss of heterozygosity (LOH)) after UV irradiation.

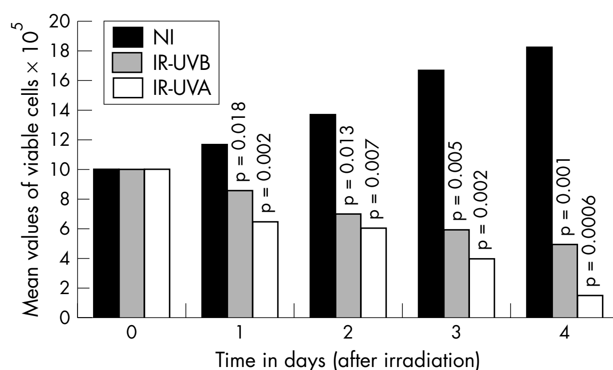

Figure 2 Summary of viability assays in ultraviolet (UV) irradiated WM35 cell lines using Trypan blue dye exclusion test. IR-UVA, UVA irradiated cells; IR-UVB, UVB irradiated cells; NI, non-irradiated.

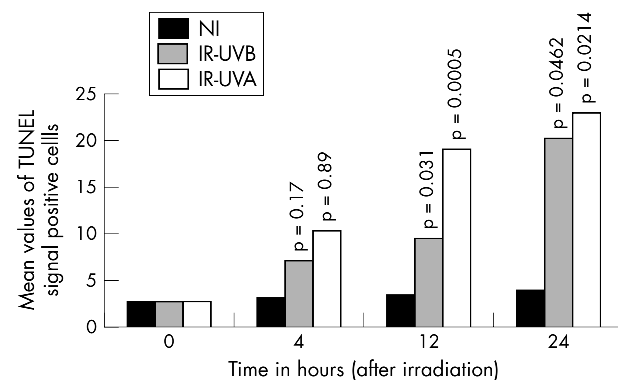

Figure 3 Semiquantitative analysis of TUNEL (terminal deoxynucleotidyl transferase mediated dUTP digoxigenin nick end labelling) positive WM35 cells after ultraviolet (UV) irradiation. IR-UVA, UVA irradiated cells; IR-UVB, UVB irradiated cells; NI, non-irradiated.

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Cell viability

Plasma membrane function and structural integrity were determined by the Trypan blue exclusion test.[19] Briefly, cell suspensions at a high concentration (10^6 cells/ml) were prepared by trypsinisation, centrifugation, and resuspension. Equal amounts (0.1 ml) of cell suspension and the Trypan blue dye were mixed thoroughly, transferred to the edge of a cover slip, allowed to run into the counting chamber of a haemocytometer, and left for one to two minutes. Both the number of stained cells and the total number of cells were then counted. The percentage of viable cells was determined by calculating the percentage of unstained cells.

Detection of apoptotic cells

To evaluate the apoptotic response after the UV exposure described above, the irradiated and non-irradiated WM cell lines were harvested at 0, 4, 12, and 24 hours after irradiation. We examined apoptosis with the terminal deoxynucleotidyl transferase mediated dUTP digoxigenin nick end labelling (TUNEL) assay before and after irradiation. The TUNEL technique was performed using the commercially available QIA33TDT-FragEL™ kit (Oncogen Research Products, Boston, Massachusetts,USA) in accordance with the manufacturer's directions. Positive controls were obtained from the manufacturer and consisted of HL-60 promyelocytic leukaemia cells. Some specimens from UV exposed WM cell lines were used as negative controls by substituting distilled water for terminal deoxynucleotidyl transferase in the protocol, as suggested by others.[20] The sections were then examined with a BH-2 Olympus microscope. The cells were then processed for evaluation by transmission electron microscopy (Tecani 10 EM and Gatan MSC with digital micrograph camera).

Selection of microsatellite markers

A panel of 21 microsatellite markers (12 markers at 1p, five at 9p, and one each at 2p, 3p, 4q, and 17p) was obtained from Research Genetics (Huntsville, Alabama, USA), and their characteristics are available online (http://www.resgen.com/products/HuMPs.php3).

DNA extraction

DNA was extracted from the cell lines using the DNeasy™ tissue kit (Qiagen, Valencia, California, USA), as described by the manufacturer.

PCR based microsatellite assay

The polymerase chain reaction (PCR) was performed in a 10 µl volume containing 1× PCR buffer (10mM Tris, 50mM KCl (pH 8.3), and 0.02% Tween 20), 0.2 µM each of the

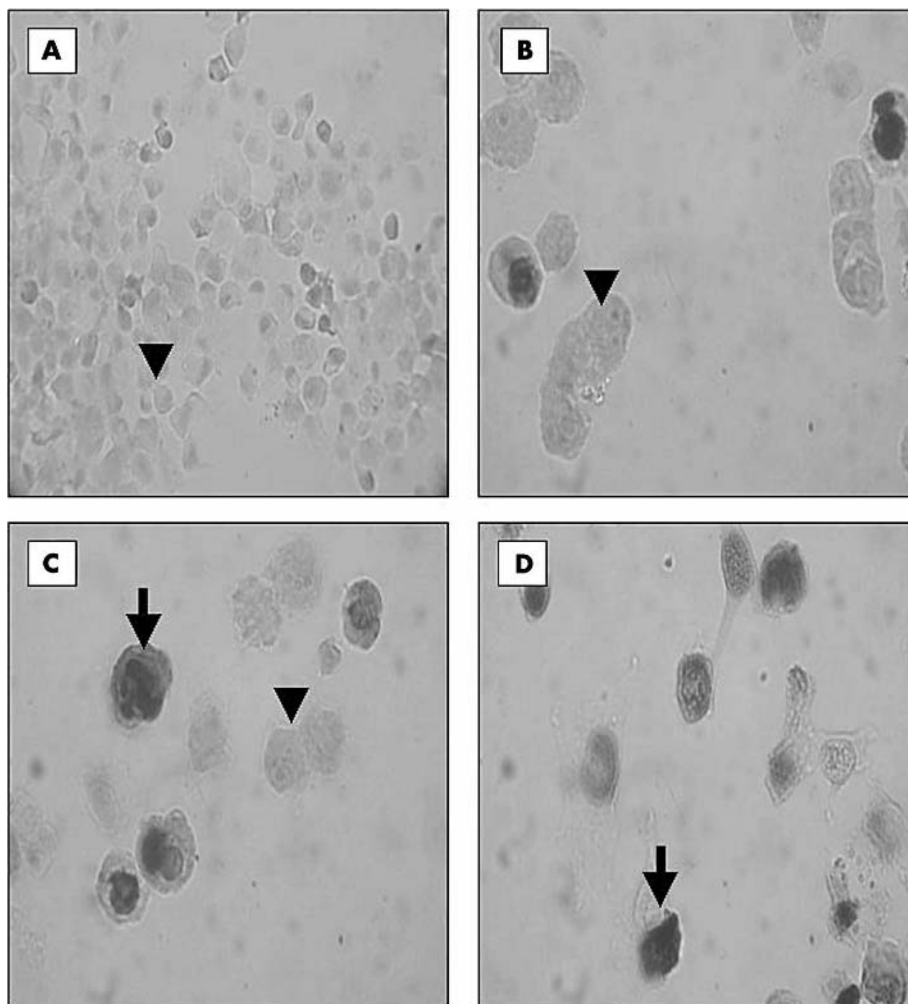

Figure 4 Apoptosis in Wistar melanoma cell lines. (A) Negative control; (B–D) apoptosis in cell lines at four, 12, and 24 hours after ultraviolet (UV) irradiation, respectively. TUNEL (terminal deoxynucleotidyl transferase mediated dUTP digoxigenin nick end labelling) signal positive (arrowhead) and negative (arrow) cells. Although both UVA and UVB induced apoptosis in the Wistar melanoma cell lines at the designated time intervals, UVA irradiation induced more apoptotic cell death than UVB irradiation.

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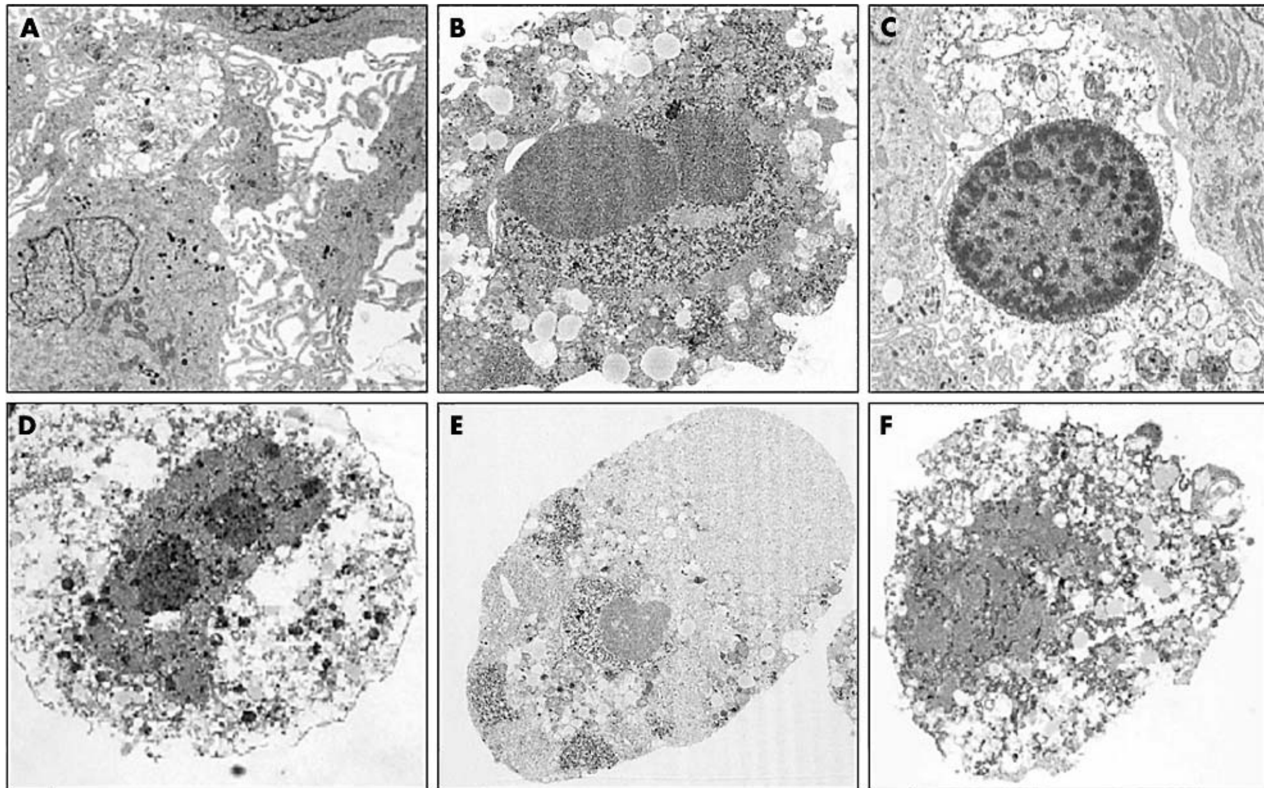

Figure 5 (A) Ultrastructural features of non-irradiated melanoma cell lines (original magnification, ×6700). Ultrastructural features of apoptosis in the irradiated Wistar melanoma cell lines included: (1) incomplete condensation of the chromatin pattern displaying a microgranular network (B; original magnification, ×4700); (2) condensed chromatin near the periphery, close to the nuclear envelope seen as one or several large homogenous masses (C and D; original magnification, ×9400 and ×49 000, respectively); (3) irregularity of the nuclear membrane (D; and E, F; original magnification, ×6700); and (4) partially disrupted chromatin as indicated by the presence of one or more nuclear vacuoles (C, E, and F).

unlabelled and ATP [γ-^{33}P] (ICN Biomedical, California, USA) labelled primers, 1 µl of DNA supernatant, 0.2 U of Taq DNA polymerase (PGC Scientific, Frederick, Maryland, USA), 125 mM each of dNTPs, and 1.5mM $MgCl_2$. PCR was performed using a ''touch down'' approach: five minutes at 94℃, followed by 36 to 38 cycles of one minute at 94℃, one minute at 56–60℃, and one minute at 72℃; with a final elongation step for nine minutes at 72℃. A ''hot start'' was also used by adding Taq DNA polymerase when the temperature in the initial ramp was > 80℃. PCR products were resolved on a 6% Long Ranger sequencing gel (FMC, Rockland, Maine, USA) and exposed to BioMax MR film (Kodak) for 24–72 hours. All PCR reactions and gel loadings were repeated at least twice, and only identical results were reported.

Assessment of MSI

MSI was defined by variations in the DNA banding pattern between irradiated and non-irradiated cell lines. Because we were working on cell lines without matching normal control DNA, only irradiated cell lines presenting with more than two alleles for each locus were considered unstable.[21] Irradiated cell lines with MSI at one or more loci were scored as MSI positive.[8] Instability at more than 30% of the tested loci was used as a cut off between MSI-L and MSI-H. Samples without MSI were scored as microsatellite stable (MSS).[8] During this assay, irradiated cell lines were simultaneously assessed for LOH. The heterozygosity loss was evaluated by comparing the intensities of the two alleles in informative cases. LOH was scored whenever the radiographic signal of one allele was absent or 50% reduced in the tumours, as determined by visual comparison of the allele intensities and use of the SciScan 5000 densitometer.

RESULTS

Effects of UV light on cell viability

The UV treated cells showed rounding up and detachment from the culture plate when compared with the control cells. Comparison of the mean values of viability among irradiated and non-irradiated cell lines revealed two facts: (1) both UVA and UVB induced pronounced cell killing within 24 hours after irradiation; and (2) UVA induced more cell killing than UVB. Similar responses were seen in all three cell lines, and fig 2 provides a summary of the viability assays in WM35. This figure shows that the mean viability was highest in non-irradiated cells, followed by the UVB irradiated cells, with the lowest values being seen in the UVA irradiated cells. The mean viability values of the non-irradiated and irradiated cell lines were significantly different, and the p values were lower for the UVA irradiated group, indicating greater significance (fig 2).

DNA fragmentation assay (TUNEL)

None of the negative controls stained with the TUNEL technique showed immunoreactivity, whereas positive TUNEL staining was seen in the positive controls. Semiquantitative evaluation of apoptosis by counting the number of TUNEL signal positive cells in both the non-irradiated and UV irradiated cell lines revealed the following two facts: (1) in the control (non-irradiated) cells, few cells showed positive signals at the designated times, whereas UV treated cells showed greatly increased numbers of positively

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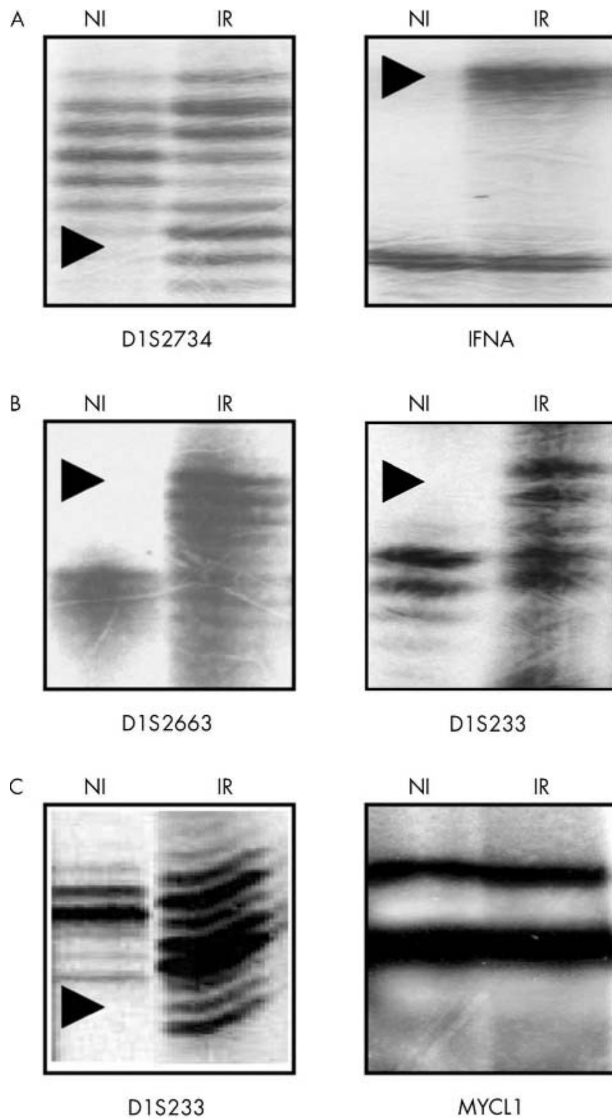

Figure 6 Microsatellite instability (MSI) in Wistar melanoma cell lines. MSI after ultraviolet (UV) B irradiation in (A) WM3211 and (B) WM35 cell lines. (C) MSI (D1S233) and microsatellite stability (MYCL1) after UVA irradiation in WM35. Instability was charactarised by the appearance of novel bands in the irradiated cell lines (arrowheads). IR, irradiated cells; NI, non-irradiated cells.

stained cells; and (2) UVA irradiation induced more apoptosis than UVB. A similar response was seen in all three cell lines, and figs 3 and 4 provide a summary of the TUNEL assay results in WM35 cells. These figures reveal that the apoptotic mean values were the highest in the UVA irradiated cells, followed by the UVB irradiated cells, with the non-irradiated cells showing the lowest values. The mean apoptotic values were significantly different between the non-irradiated and irradiated cell lines, with the p values being lower in the UVA irradiated group, indicating greater significance (fig 3). Positive TUNEL results are indicative, but not proof of, the presence of apoptotic cells, because there are several forms of programmed cell death that can induce DNA degradation in the absence of apoptotic caspase activation. Therefore, the apoptotic activity in our series was also confirmed by the ultrastructural detection of characteristic apoptotic patterns in the irradiated WM cell lines. These features included: (1) incomplete condensation of the chromatin pattern, displaying a microgranular network (fig 5B); (2) condensed chromatin near the periphery, close to the nuclear envelope, seen as one or several large homogenous masses (fig 5C, D); (3) irregularity of the nuclear membrane (fig 5D–F); and (4) partially disrupted chromatin, as indicated by the presence of one or more nuclear vacuoles (fig 5C, E, F).

Frequency of MSI after UVR

MSI was detected in the WM35 and WM3211 cell lines after UVR, but not in WM1650, in duplicate sets of experiments. The allelic pattern in irradiated cells was characterised by the presence of novel bands above or below those present in non-irradiated cells (fig 6). MSI was found at four loci in the WM35 cells (D1S2734, D1S233, MYCL1, and IFNA) after UVB irradiation and at one locus (D1S233) after UVA irradiation. In the WM3211 cells, MSI was seen after UVB irradiation only, and was found at five loci (D1S2663, D1S233, D1S513, D9S171, and TP53). Overall, the most frequently affected locus was D1S233. In keeping with the MSI-L pattern reported previously by us in excised melanocytic dysplastic naevi and CMM,[8] the frequency of MSI ranged from 0.0% to 19.0% and from 0.0% to 4.7% after UVB and UVA irradiation, respectively (table 1).

DISCUSSION

Previous studies of excised melanocytic skin tumours revealed the involvement of UVR, apoptosis, and a gradual increase in MSI, which correlated with the progression and increased aggressiveness of these tumours.[22–24] Based on these studies, we hypothesised that UVR contributes to the molecular pathogenesis of CMM arising on sun-exposed skin by causing MSI, which results in mutations in genes involved in neoplastic transformation and tumour progression. To test our hypothesis, we exposed radial growth phase CMM cell lines to UVA and UVB and examined them for viability, the presence of apoptosis, and MSI using TUNEL and PCR based microsatellite assays.

In agreement with other studies, our data revealed that UVR (in the range capable of causing sunburn in vivo) generally induces cell death and a drastic increase in the apoptotic activity in the irradiated WM cell lines,[25–28] suggesting excessive underlying DNA damage beyond the capacity of the repair system.[29] This DNA damage may be either in the form of accumulated pyrimidine dimers (induced by UVB) or oxidatively modified bases (induced by UVA). The accumulation of these products as a possible mechanism for the apoptosis seen in WM cell lines concurs with the apoptotic changes induced by UVA and UVB.[28 30] Because DNA absorbs little, if any, UVA, the genotoxicity of UVA is mostly the result of the generation of reactive oxygen radicals,[6] which have a definitive role in apoptosis.[31] In support of this principle, exposure to H_2O_2 is a potent inducer of apoptosis in HL-60 cells,[32] and oxygen radical scavengers can block DNA nuclear fragmentation and the formation of apoptotic bodies after UVA irradiation.[28 33]

''Ultraviolet irradiation generally induces cell death and a drastic increase in the apoptotic activity in irradiated Wistar melanoma cell lines, suggesting excessive underlying DNA damage beyond the capacity of the repair system''

In vivo, melanoma cells are traditionally thought to be highly radioresistant. However, several factors may have contributed to the response of the melanoma cells to UVR seen in our study. First, the radioresistance of melanoma cells was noted in an in vivo system, where skin microenvironmental factors probably influence the cells and contribute to

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Table 1 Microsatellite instability in Wistar melanoma (WM) cell lines

		UVB			UVA		
Chromosome	Marker	WM35	WM1650	WM3211	WM35	WM1650	WM3211
1p36.33	D1S243	NA	NA	MSS	MSS	MSS	MSS
1p36.33	D1S468	MSS	MSS	NA	MSS	MSS	MSS
1p36.32	D1S214	NA	NA	NA	NA	NA	NA
1p36.32	D1S2663	MSS	MSS	MSI	MSS	MSS	MSS
1p36.32	D1S2642	MSS	NA	MSS	MSS	MSS	MSS
1p36.23	D1S489	MSS	MSS	MSS	NA	NA	NA
1p36.23	D1S228	MSS	MSS	MSS	MSS	NA	MSS
1p35	D1S2734	MSI	MSS	MSS	MSS	MSS	MSS
1p34	D1S513	MSS	MSS	MSI	NA	NA	NA
1p34	D1S233	MSI	MSS	MSI	MSI	NA	MSS
1p34	D1S2832	MSS	MSS	MSS	NA	NA	MSS
1p33	MYCL1	MSI	MSS	MSS	MSS	NA	MSS
2p	BAT-26	MSS	MSS	MSS	NA	NA	NA
3p	BAT40	MSS	MSS	MSS	NA	NA	NA
4q12	BAT-25	MSS	MSS	MSS	MSS	NA	MSS
17p	TP53	MSS	MSS	MSI	MSS	NA	MSS
9p22	D9S162	NA	NA	MSS	NA	NA	NA
9p22	IFNA	MSI	MSS	MSS	NA	NA	MSS
9p21	D9S171	NA	NA	MSI	NA	NA	NA
9p21	D9S126	NA	NA	NA	NA	NA	NA
9p21	D9S169	NA	NA	NA	NA	NA	NA

MSI, microsatellite instability; MSS, microsatellite stable; NA, non-amplified; UV, ultraviolet.

this resistance. The in vitro system used here is not affected by changes in the skin microenvironment after UV exposure. Second, ionising rather than non-ionising irradiation (UVR) has been used in the radiotherapy of melanoma.

The MSI-L pattern found in irradiated WM cell lines is in keeping with other studies reporting similar changes in excised melanocytic lesions.[8 22] This finding also supports our hypothesis that UVR can induce MSI in these lesions, suggests that MSI can arise early in melanoma pathogenesis, and suggests that UVR may be a driving force in the evolution of at least some CMMs, such as those arising on sun exposed skin. Because MSI may affect the function of the genes in which the microsatellites reside, the presence of MSI in the markers flanking the melanoma susceptibility genes in the 1p and 9p regions warrants further mutational analysis of these genes. Interestingly, UVB produced more MSI than UVA. In this regard, because UVB penetrates less deeply than UVA, the greater effect of UVB on MSI may help explain why most CMMs begin superficially in or near the epidermis.

We suggest three possibilities to support our hypothesis and to explain how UVR was able to induce MSI in the irradiated WM cell lines. First, the presence of MSI after a single exposure to UVR may merely represent a hypermutability state in these cell lines. In this regard, because exposure to UVR in vitro represents a dramatic change in the environment of WM tumour cells, it is conceivable that the cells acquire selective advantages by generating additional mutations, perhaps in the microsatellite repeats, to adjust to the in vitro conditions.[34]

Second, the presence of MSI after UV exposure may reflect underlying DNA damage by reactive oxygen species. Because UVR inhibits free radical scavenging activity[35] and inactivates antioxidant enzymes,[5 36] it enhances DNA damage by reactive oxygen radicals. The generation of these radicals is estimated to be responsible for 20 000 hits to DNA/cell/day,[37–40] representing a major source of mutations. The role of oxidative stress in melanoma tumorigenesis is supported by three observations, namely: (1) oxygen radical induced DNA damage is involved in some tumours, such as breast cancers[41]; (2) CMM cell lines constitutively produce and accumulate hydrogen peroxide at levels comparable to activated polymorphonuclear lymphocytes[42]; and (3) exposure of DNA to reactive oxygen species in vitro can induce MSI,[43] even in the absence of MMR gene defects, by several mechanisms. One of these mechanisms involves the retardation of DNA polymerase at sites of oxidative base damage, with subsequent strand displacement and misalignment of the growing strands at different positions on the template. Furthermore, strand scission within the microsatellite repeat sequences by reactive oxygen species enhances the possibility of strand misalignment, with the subsequent generation of insertion or deletion loops. The reactive oxygen radicals can also induce single strand breaks in the microsatellite sequences, increasing the formation of slipped strand intermediates.[44]

The third possibility is that the presence of MSI after UV exposure may reflect underlying DNA replication infidelity. As a part of the response to the UV irradiation of WM cell lines, there is accumulation of damaged DNA photoproducts that stimulate the induction of stress induced secreted proteins in the cell lines.[45] Subsequently, these proteins promote activation of the genes encoding extracellular proteins,[46] cytokines,[47] and several yet unidentified proteins. These three protein classes are also involved in inducing replication infidelity, and may favour genetic instability and oncogenic transformation.[4 48] In support of this concept, the secretion of these proteins was found to induce genetic instability in mouse T lymphoma cell lines after UVR,[4] and

Take home messages

- Ultraviolet (UV) irradiation induced pronounced apoptosis in radial growth phase melanoma cells, with UVA having a greater effect than UVB
- UV also induced microsatellite instability (MSI) and was more prevalent after UVB than UVA irradiation
- Thus, the ability of erythemogenic UV irradiation to induce both apoptosis and MSI in radial growth phase melanoma cells suggests that it plays a role in the pathogenesis of melanoma
- This instability may reflect a hypermutability state, oxidative stress induced DNA damage, replication infidelity, or a combination of these factors

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some of these proteins are constitutively expressed in several cancer prone syndromes[49] and tumour cell lines.[50]

The absence of LOH after UV irradiation in WM cells is in agreement with previous studies,[51] and suggests that the initial genetic events after UVR do not include LOH at an early stage. This observation is in accordance with the fact that LOH is usually associated with exposure to ionising irradiation but not to non-ionising irradiation.[52]

Of note, further experiments on the same cell lines are suggestive of increased malignant potential. In this regard, UVR was able to induce both point mutations in the mismatch repair genes[53 54] and ultrastructural changes in keeping with malignant changes. However, determining whether the cells that survive UVR have in fact an increased tumorigenic potential relative to the parental ones (for example, growth as xenografts in immunosuppressed mice) will require additional experiments. The results of our present study will be useful to compare and contrast with such subsequent investigations.

To the best of our knowledge, our study is the first to suggest the induction of apoptosis and MSI in WM cell lines after exposure to UVR. However, whether MSI reflects a hypermutability state, oxidative stress induced DNA damage, replication infidelity, or a combination of these three must be determined by further investigations.

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