Apoptosis and cytokine release induced by ionizing or ultraviolet B radiation in primary and immortalized human keratinocytes

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We have compared the induction of apoptosis and cytokine release by UVB and γ-radiation in primary (untransformed) and in two immortalized human epithelial/keratinocyte cell lines, HaCaT and KB (KB is now known to be a subtype of the ubiquitous keratin-forming tumour cell line HeLa and we therefore designate it HeLa-KB). In both the primary and the immortalized cell lines apoptosis and release of the inflammatory cytokine interleukin-6 are induced rapidly following UVB irradiation. In contrast, only the immortalized cells undergo apoptosis and release interleukin-6 after γ-irradiation and here the onset of apoptosis and cytokine release are delayed. The same distinction between primary and immortalized cells was observed when double-strand breaks were induced with the anticancer drug mitoxantrone, which stabilizes topoisomerase II cleavable complexes. We suggest that immortalization may sensitize keratinocytes to the apoptogenic effect of ionizing radiation or mitoxantrone by deregulating normal cell cycle checkpoints. In both human keratinocytes and fibroblasts, cell killing, as assayed by loss of colony-forming ability, is not coupled to apoptosis. Immortalization increases resistance to γ-radiation killing but sensitizes to apoptosis. In contrast, although immortalization also sensitizes to UVB-induced apoptosis, it does not affect UVB-induced cell killing. Apoptosis unambiguously indicates death at the single cell level but clonal cell survival integrates all the cellular and genetic processes which prevent or permit a scorable clone to develop.

Abbreviations: DSB, double-strand break; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-6, interleukin-6; NHEK, normal human epidermal keratinocytes; RT–PCR, reverse transcription–PCR; TGFβ, transforming growth factor-β; TNFα, tumour necrosis factor α; VDM, time lapse video microscopy.

Introduction

Studies of ionizing and ultraviolet (UVC, 254 nm) radiation-induced DNA damage and repair in human cells have largely focused on the response of primary (untransformed) fibroblast cell lines (1,2). We have used three classes of human cell in this study. (i) We describe as ‘primary’ or ‘untransformed’ unmodified cells maintained as a cell line which has been subjected to no form of immortalization or transformation. The normal human epidermal keratinocytes (NHEK) used were derived from normal human foreskin epidermis. (ii) We have used a tumour-derived human epithelial keratin-forming cell line (HeLa-KB) which is both transformed and immortal. (iii) Finally, we have used a human keratinocyte cell line (HaCaT), which is immortalized but is not tumorigenic (3,4). Both HeLa-KB and HaCaT will be referred to here as immortalized cells. We realise that the term primary has been proposed to be restricted to material which has not been subcultured (5) and that it would be possible to describe HaCaT cells as untransformed. There is the danger of a situation akin to that envisaged by Orwell (6), where it is not possible to use the recommended definitions of terms to describe what one means. Primary fibroblasts are resistant to γ-radiation-induced apoptosis, but transformation with SV40 T antigen increases the susceptibility of the cells to this mode of death, particularly when the cells are derived from patients suffering from the DNA repair defect syndrome ataxia telangiectasia (7–10). Paradoxically, we and others have demonstrated that the immortalization process substantially reduces γ-radiation-induced cell killing, as assessed by clonogenic assays (11). In contrast, immortalization has no differential effect on clonal cell survival following exposure to UVC (12). Primary fibroblasts are also largely refractory to apoptosis in response to UVB radiation (UVB, 280–315 nm) (E.Capulas and C.F.Arlett, unpublished observations), but again their SV40-transformed, immortalized derivatives do undergo apoptosis. This implies an uncoupling of cell lethality and apoptosis and suggests that any equating of apoptosis between SV40-transformed and untransformed fibroblasts should be viewed with caution. An effect of transformation on apoptosis is also found in thyroid cells, where irradiation with up to 8 Gy induces apoptosis in transformed but not in primary cells (13). However, whether this effect of immortalization/transformation is true for other cell types is not known.

In this paper we have investigated responses to UVB, since this is the major DNA-damaging component of solar radiation, rather than the shorter wavelength UVC used in most laboratory studies, which does not reach the Earth’s surface (14). The main physiologically relevant target cells for UVB-induced non-melanoma skin cancer are keratinocytes (15). Thus, the response of this class of cells is most relevant to an understanding of the contribution of apoptosis to solar carcinogenesis. Sunburn cells bear all the characteristics of apoptotic keratinocytes (16), indicating that solar radiation is able to induce apoptosis in vivo (17,18). There are a number of studies which
reveal that UV radiation is proficient in inducing apoptosis in primary or immortalized human keratinocytes in culture (19–24).

Ionizing radiation and UV-induced DNA damage have been shown to initiate the expression of various circulatory cytokines such as interleukin-6 (IL-6) (25–27) and some of these responses may be related to apoptosis. Thus Iglesias et al. (28) have demonstrated that in keratinocytes derived from the cervix and infected with a viral protein the extent of apoptosis correlates with the amount of interleukin-1α released. IL-6 itself is a pluripotent cytokine which is involved in acute pro-inflammatory processes associated with overexposure to ionizing radiation (29) and UV (30). Increases in IL-6 levels in the serum are associated with the development of fever and synthesis of acute phase proteins in the liver (30–32). Cyclobutane pyrimidine dimers induced by UVB have been shown to cause the release of both IL-6 (27) and interleukin-10 (33). Although cytokine release is induced by UV in a similar way in both immortalized and primary keratinocytes (27), the effect of ionizing radiation on cytokine release in these cells has not been resolved. However, Santin et al. (34) have shown that induction of the release of transforming growth factor β (TGFβ) by ionizing radiation is inhibited in freshly isolated ovary cells, but greatly enhanced in established ovarian tumour cell lines.

In this study we have compared the biological responses of primary dermal keratinocytes (NHEK) and two immortalized cell lines. HaCaT cells are of dermal keratinocyte origin (3) and represent a direct comparison with NHEK. HeLa-KB is a keratin-forming cell line (35) which was originally designated KB and thought to be derived from an oral tumour, but has now been shown to be HeLa (35,36). HeLa is a ubiquitous human epithelial cell line of cervical origin and is the original immortal human cancer cell line (37). We have sought to compare the effects of two different classes of DNA damage: complete growth medium for 7 (33). Although cytokine release is induced by UV in a similar way in both immortalized and primary keratinocytes (27), the effect of ionizing radiation on cytokine release in these cells has not been resolved. However, Santin et al. (34) have shown that induction of the release of transforming growth factor β (TGFβ) by ionizing radiation is inhibited in freshly isolated ovary cells, but greatly enhanced in established ovarian tumour cell lines.

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Materials and methods

Cell culture

NHEK derived from neonatal foreskin were obtained from Clonetics (San Diego, CA) and were maintained in complete Keratinocyte Basal Medium (Clonetics) as described elsewhere (27). In cytokine assays, incubation before and after irradiation was in this medium with omission of hydrocortisone. Human HaCaT keratinocytes (kindly provided by Dr P.Boukamp, DKFZ, Heidelberg, Germany through Dr C.Mathersill, Dublin, Republic of Ireland) were cultured in Dulbecco’s modified Eagle’s medium/Ham’s F-12 nutrient mixture with 20 U/ml penicillin, 20 µg/ml streptomycin, 2 mM glutamine, 15 mM HEPES, 0.5 µg/ml hydrocortisone and 10% foetal calf serum.

KB cells were obtained from Professor J. Knuthmann (Düsseldorf, Germany) and originated from the ATCC (Rockville, MD). This cell line was originally believed to be derived from an epidermal tumour of the mouth, but has now been shown to be HeLa (35,36). We have recently confirmed this identification as HeLa by simple tandem repeat profiling (Universal Diagnostics Ltd; Masters et al., in preparation) and copies of the relevant profile are available from the corresponding author. HeLa represents a keratin-forming human tumour cell line of cervical epithelial origin (37). Despite its apparent basis in HeLa contamination (35,41; Masters et al., in preparation), KB is still commonly presented as a separate entity, with over 380 papers referring to KB cells listed in Medline since the link to HeLa was discovered (36). We will therefore refer to these cells as HeLa-KB throughout this paper, a terminology which is consistent with that for other HeLa sublines and which retains a link to the external literature on KB cells. The HeLa-KB cells were maintained in monolayer cultures in Eagle’s minimum essential medium with 20 U penicillin per ml, 20 µg/ml streptomycin, 2 mM glutamine and 10% foetal calf serum (Gibco, Paisley, UK).

All cultures were maintained at 37°C in a humidified atmosphere containing 5% CO2. Concentrations of calcium (which may affect keratinocyte apoptosis) (21,42) in each medium were: Keratinocyte Basal Medium, 0.15 mM; Eagle’s minimum essential medium, 1.5 mM; Dulbecco’s modified Eagle’s medium/ Ham’s F-12, 1.05 mM.

UVB irradiation was performed as described previously (27). For γ-irradiation, 1×106 HaCaT, 8×105 HeLa-KB or 1.5×106 NHEK cells were cultured overnight in the appropriate medium (NHEK in medium without hydrocortisone when assaying for cytokines) in tissue culture grade 3.5 cm Petri dishes (Nunc, Roskilde, Denmark). The cells were irradiated in culture medium, which was then immediately replaced with fresh medium after treatment. For treatment with mitoxantrone (Lederle Laboratories, Gosport, UK), cells in dishes were incubated with concentrations of 25, 50 and 100 ng/ml for 48 and 72 h. To measure IL-6 release supernatants were collected 24 h after irradiation and kept at −20°C until determination of cytokine concentration. Time course studies showed this to be the optimal interval (data not shown). For the assessment of apoptosis using the Apoptag® kit, cells were cultured in medium containing hydrocortisone, trypsinized at various times after treatment, spun onto Teflon-coated slides, air dried and then kept at −20°C until staining for apoptotic cells. To perform VDM, dishes were transported from the MRC Cell Mutation Unit to the ICRF Videomicroscopy Unit in London. This normally took ~3 h from treatment to commencement of microscopy. The dishes were carried in a polystyrene block to protect the cells from temperature extremes but no attempt was made to maintain temperature or pH during transportation of the material.

For clonogenic assays, appropriate numbers of cells were seeded in 3.5 cm dishes, allowed to adhere overnight and shown to still be in the single cell state. They were then irradiated as described above and incubated in fresh complete growth medium for 7–11 days. Colonies were stained with methylene blue, counted (a surviving colony is defined as one consisting of ≥50 cells) and the surviving fraction calculated.

Irradiation sources

UVB irradiation was performed through the bottom of the culture dish using a bank of four Westinghouse broad-band FS20 sunlamps (38,43). Fluence rates were determined with an International Light radiometer (IL1350) and (VDM). These were exploited to validate and calibrate each were typically 5 W/m2 for UVB. Other. In parallel, we assessed the cytotoxic effects of ionizing or UVB irradiation by a clonogenic assay. This assay measures cellular reproductive capacity, i.e. the ability to divide and form viable colonies. However, it does not always necessarily reflect the ability of a particular cell to undergo apoptosis (5). We also took this opportunity to investigate whether cellular immortalization affected the induction of cytokine release by UVB or γ-irradiation in human keratinocytes.

Detection of apoptotic cells

Apoptotic cells were detected using the Apoptag® kit (Oncor, Gaithersburg, MD) according to the supplier’s instructions. The free 3’-OH ends generated by DNA fragmentation in apoptotic cells were end-labelled with fluorescein-conjugated anti-digoxigenin antibody (green fluorescence). The DNA was counterstained with propidium iodide (red fluorescence) and the apoptotic cells were scored by fluorescence microscopy.

Detection of apoptosis was also performed using VDM. Irradiated or control cells were grown in 3.5 cm dishes in an enclosed 5% CO2 atmosphere containing 95% air. Images were acquired every 2 min for ~72 h using high resolution CCD cameras (Sony M370CE) in conjunction with video recorders (Sony PWV-2800P) driven by an animation controller (Eos Electronics, UK). For each film, 100 cells were observed and the timing of each apoptotic event recorded. Scores of >100 are feasible if division occurs during the experimental period.

Determination of cytokine concentrations

Concentrations of IL-6 were determined by enzyme-linked immunosassay as described in detail elsewhere (27).

Reverse transcription–PCR (RT–PCR)

RNA was extracted using Trizol (Gibco, Paisley, UK) according to the supplier’s instructions. An aliquot of 1 µg RNA was used to synthesize cDNA using a first strand cDNA synthesis kit (Pharmacia, St Albans, UK). PCR was performed as described previously (27).
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Fig. 1. Percentage of apoptotic cells (± SEM) as determined with Apoptag®
24 h (■), 48 h (cross-hatched square) and 72 h (stippled square) after the
indicated dose of γ-radiation. (A) NHEK; (B) HeLa-KB; (C) HaCaT. All
results are means of three independent experiments.

Fig. 2. Percentage of apoptotic cells (± SEM) as determined with Apoptag®
24 h (■) after the indicated dose of UVB radiation. (A) NHEK; (B) HeLa-
KB; (C) HaCaT. All results are means of three independent experiments.

performed as described previously, with the minor modification that the IL-6
and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) fragments were
amplified simultaneously in the same reaction mixture. PCR products (10 µl)
were electrophoresed on a 2% agarose gel which was stained overnight with
Vistra Green according to the supplier’s instructions (Amersham, St Albans,
UK). Quantification of the PCR products was performed using a STORM
phosphorimager (Molecular Dynamics, Chesham, UK). The level of IL-6
mRNA expression was expressed as the ratio of the intensity of the IL-6 PCR
product to the corresponding GAPDH PCR product and normalized to the
unirradiated sample.

Results

Apoptosis assessed by DNA 3'-OH end-labelling

Apoptosis induced by γ-radiation and UVB in primary and
immortalized keratinocytes. γ-Irradiation did not induce
apoptosis in the primary keratinocytes even at doses of up to
20 Gy (Figure 1A). This is in contrast to the results obtained
with the two immortalized cell lines (Figure 1B and C), where
higher doses were effective in inducing apoptosis at 48 and
72 h (HaCaT) or 72 h (HeLa-KB). In additional experiments
of the same design (data not shown) the number of apoptotic
cells in NHEK and HeLa-KB cells was followed for up to
6 days. Five days after irradiation with 20 Gy, 100% of the
HeLa-KB cells had undergone apoptosis, while the same dose
gave rise to only negligible levels of apoptosis in NHEK cells.
The NHEK cells did not divide, but since RNA and protein
synthesis continued, they increased slowly in size. The response
is similar to that of primary fibroblasts, where we have
observed that the irradiated population remains intact for up
to 2 weeks (C.F.Arlett and E.Capulas, unpublished).

The three cell lines were also irradiated with various doses of
UVB and apoptosis was scored 24 h after irradiation; in
this case both primary and immortalized cells underwent
apoptosis (Figure 2). HeLa-KB cells exhibited greater sensitiv-
ity to UVB-induced apoptosis than NHEK primary cells or
HaCaT cells.

Apoptosis induced by mitoxantrone in primary and immortal-
ized keratinocytes. Since primary keratinocytes proved to be
refractory to the induction of apoptosis by γ-irradiation, we
next investigated the effect of the DNA topoisomerase II
inhibitor mitoxantrone as an alternative means of generating
DNA DSB (40). As shown in Figure 3, a pattern similar to
that seen with ionizing radiation was obtained: the immortalized
cells underwent apoptosis and the primary keratinocytes did
not. As with γ-radiation, the onset of apoptosis was late.

Apoptosis assessed by VDM

VDM was used to complement and confirm our observations
with the Apoptag® assay. VDM generates a cumulative record
of apoptosis in a particular cell population over 72 h. As
shown in Figure 4, NHEK again proved not to be sensitive to γ
radiation-induced apoptosis, but responded to UVB. Apoptosis
was induced in both immortalized keratinocyte cell lines by
both treatments. Clearly UVB-induced apoptosis in HeLa-KB
cells is a very early event, while the first apoptotic events
following γ-irradiation were not detected until ≥40 h after
exposure.
The mitoxantrone results were also confirmed by VDM (Figure 4D). Both HeLa-KB and HaCaT cells are susceptible to apoptosis as a late event, but no apoptosis was recorded in the primary cell population over a 72 h period.

**Cell killing**

The cytotoxic effects of \(\gamma\)-irradiation and UVB were evaluated by clonogenic survival. Differential sensitivity to \(\gamma\)-irradiation was seen, NHEK cells proving the most sensitive and HaCaT cells the most resistant (Figure 5A). With UVB there was no difference in sensitivity between the three cell cultures (Figure 5B).

**Cytokine induction**

DNA damage can lead to the secretion of a large array of cytokines in human and murine keratinocytes. Ionizing radiation did not induce IL-6 release in primary keratinocytes (Figure 6A). In contrast, doses >6 Gy initiated the release of the cytokine 72 h after irradiation in HeLa-KB and HaCaT cells (Figure 6B and C). When the high constitutive level of IL-6 in HeLa-KB cells is taken into account, the proportional increase in the two cell lines was comparable. Following irradiation with UVB, the dose–response characteristics for cytokine release were similar for all cell types, with a maximum observed at 642 \(\text{J/m}^2\) (Figure 7). However, the proportional increase in IL-6 secretion was greater in NHEK and HaCaT cells than in HeLa-KB cells.

In order to investigate the effect of the treatments on IL-6 mRNA levels, HeLa-KB and NHEK cells were irradiated with doses of 20 Gy \(\gamma\)-radiation or 642 \(\text{J/m}^2\) UVB. After intervals of 8, 24 and 48 h mRNA was extracted and RT–PCR performed. The results of a representative experiment are presented in Figure 8. A small induction of IL-6 mRNA was observed in \(\gamma\)-irradiated HeLa-KB but not NHEK cells 8 h after treatment. Irradiation with UVB led to a more pronounced increase in IL-6 mRNA expression with both NHEK and HeLa-KB cells. Similar observations were made 24 and 48 h after irradiation.

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**Fig. 3.** Percentage of apoptotic cells (± SEM) as determined with Apoptag® 48 (cross-hatched square) and 72 h (stippled square) after the indicated dose of mitoxantrone. (A) NHEK; (B) HeLa-KB; (C) HaCaT. All results are means of three independent experiments.
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Fig. 5. Survival of primary and immortalized human keratinocytes following (A) γ-irradiation and (B) UVB irradiation. NHEK, ●; HeLa-KB, △; HaCaT, □. All curves are the means of three independent experiments.

Fig. 6. IL-6 release by primary and immortalized human keratinocytes following γ-irradiation. (A) NHEK; (B) HeLa-KB; (C) HaCaT. IL-6 levels were determined 24 (■), 48 (cross-hatched square) and 72 h (stippled square) after the indicated dose of γ-radiation. Values are the means of three independent determinations.

Fig. 7. IL-6 release by primary and immortalized human keratinocytes following UVB irradiation. (A) NHEK; (B) HeLa-KB; (C) HaCaT. IL-6 levels were determined 24 h after irradiation. Values are the means of three independent determinations.

Discussion

The primary objective of this study was to ask whether cell immortalization could influence the apoptotic response and cytokine release resulting from different classes of DNA damage in human keratinocytes. To this end, immortalized and primary keratinocytes were treated with ionizing radiation or the topoisomerase II inhibitor mitoxantrone, both of which induce primarily DSB (40,44), or UVB radiation, which induces a range of excisable photoproducts (38,43). The apoptotic response was evaluated using two different methods: the 3′-OH-labelling of fragmented DNA, which scores the proportion of apoptotic cells at a particular time, and VDM, which observes the fate of a cell population over 72 h. Both methods demonstrated induction of apoptosis in the primary keratinocytes and in the immortalized cell lines following UVB irradiation and in the immortalized cells following γ-irradiation or mitoxantrone treatment. In addition, both methods provided no evidence of apoptosis in primary...
keratinocytes following γ-irradiation or mitoxantrone treatment. Since primary keratinocytes can undergo apoptosis following UVB irradiation, an apoptotic programme must be intact in these cells.

When we compared results obtained with both procedures for measuring apoptosis a significant correlation ($P < 0.001$) was obtained (Figure 9). The VDM protocol had the potential to generate higher scores, reflecting the cumulative nature of these determinations. It also generated lower background levels, when no induction of apoptosis was anticipated. The highly significant correlation should give confidence in results obtained with either assay system used singly.

The results with primary human material are not specific to the neonatal NHEK cells used in this study. Keratinocytes grown out from fragments of skin biopsies from two normal adult individuals have shown similar levels of UVB-induced apoptosis to those found here with NHEK cells (C.Petit-Frère, C.F.Arlett and E.Capulas, in preparation) and, in one limited experiment, keratinocytes from a skin fragment did not show ionizing radiation-induced apoptosis (unpublished).

Apoptosis induced by UVB occurred more rapidly than that induced by γ-radiation, suggesting that different mechanisms are involved. The time to onset of apoptosis as revealed by VDM for HeLa-KB cells showed that UVB-induced damage is expressed very early while γ-radiation-induced damage may take 2 or more days before it results in apoptosis. γ-Radiation-induced apoptosis was similarly delayed in HaCaT cells, implying a cell cycle dependence as well as lesion specificity for apoptosis. Indeed, work from Weller et al. (45) has demonstrated that in human keratinocytes, treatment with caffeine overrides the G2 delay in γ-irradiated cells but has little effect on the perturbation of the cell cycle induced by UVB, clearly indicating different signal transduction pathways from these different types of DNA damage.

Lesion specificity in the response of the three cultures was confirmed by use of the anticancer drug mitoxantrone (40). Mitoxantrone generates DNA DSB by stabilizing topoisomerase II cleavable complexes and, as anticipated, the cellular response to this agent mirrored that for γ-radiation. With both Apoptag® and VDM a positive response for immortalized KB and HaCaT cells and a negative response for the primary keratinocytes was observed. Greenwood et al. (46) reported that an alternative topoisomerase II inhibitor, etoposide, induced apoptosis earlier and more efficiently in a human lymphoblastoid cell line TK6 with normal p53 in comparison with a cell line WI-L2-NS with mutant p53. These results were mirrored by the induction of apoptosis by ionizing radiation in the same pair of immortalized cell lines (47).

In contrast, our study does not suggest any simple correlation of expression of p53 with increased susceptibility to apoptosis. Primary keratinocytes should have normal expression of p53, whereas HaCaT cells are double allelic mutants in the gene (48). Although many HeLa sublines show low expression of p53, Mogi et al. (49) have reported constitutive expression in ‘KB’ cells and we have confirmed this observation in our HeLa-KB cells (data not shown), although we have no evidence
whether or not there is functional activity. The level of p53 protein in HeLa-KB was neither increased nor decreased by γ-irradiation, but 2 h after UVB irradiation p53 levels appeared to be reduced and after 24 h levels were clearly increased (unpublished).

Results from other studies emphasize the marked differences in the apoptotic response between primary and immortalized cells. The avian anaemia virus protein known as apoptosis is able to induce apoptosis only in immortalized keratinocytes or fibroblasts (50). TRAIL, the tumour necrosis factor-related apoptosis-inducing ligand, is reported to induce apoptosis in “KB” and HaCaT but not in primary keratinocytes (51). Of particular relevance is the observation that in immortalized keratinocytes which are either p53 mutant (HaCaT) or p53 wild-type (SC11) UVB radiation is able to activate a CD95-dependent pathway in the absence of ligand, indicating that UVB is able to initiate apoptosis independently of the involvement of p53 (24). The existence of both p53-dependent and p53-independent pathways in the response of human keratinocytes to UVB irradiation is confirmed by the study of Gniadecki et al. (22). This study and that of Benassi et al. (21) are consistent in revealing that UVB-induced apoptosis is an early event.

We next undertook a comparison of the lethal effects of the two forms of radiation using a clonogenic assay. With γ-radiation the primary NHEK cells proved substantially more radiosensitive than the HeLa-KB or HaCaT cells, an observation consistent with our experience of the influence of immortalization on cell survival in fibroblasts (11). The relative resistance of p53 mutant HaCaT cells (48) supports observations made with p53 mutant fibroblasts from Li–Fraumeni patients (52). Primary p53 wild-type human fibroblasts undergo a permanent G0/G1 arrest after γ-radiation while p53 mutant cells do not arrest regardless of the dose (53,54). HeLa-KB cells express p53 protein (49), but it may or may not be functional. It is possible that the different biological responses of immortalized and primary keratinocytes to ionizing radiation may correspond to the ability or inability to activate cell cycle checkpoints, though not necessarily via p53. In our experiments we were not able to discriminate between the three cultures with respect to their sensitivity to UVB radiation.

Our data suggest an uncoupling of clonal cell survival and apoptosis. Thus for γ-radiation the radiosensitive NHEK cells were refractory to induction of apoptosis. For HeLa-KB and HaCaT cells, 10 Gy of γ-irradiation generated 10–15% apoptosis and more than 95% loss of clonogenic survival. With UVB radiation there was massively more lethality than apoptosis at a dose of 642 J/m², even in the HeLa-KB cells, which were most susceptible to UVB-induced apoptosis. Olive and Durand (55) have reviewed the data for radiosensitivity and apoptosis and conclude that although in general they are correlated, this is not consistent. In their survey, the number of studies where both end-points were measured at the same time were limited and there was a tendency in some studies to compare primary and immortalized cells or even different cell types. Interestingly, Levine et al. (56) have found that a high level of spontaneous apoptosis in cervical tumours is associated with poor prognosis and response to radiotherapy. Clonal survival integrates but does not enumerate all the events which contribute to cell death. Thus, for example, segregation of lethal events in cell division can lead to a smaller but viable colony. We suggest that, with the exception of the response of the NHEK cells to γ-radiation where the uncoupling is absolute, the resolution of any correlation must depend upon more detailed analysis, perhaps involving single cell dissection of developing clones.

Another consequence of radiation-induced DNA damage is the release of various cytokines. These cytokines are thought to play important roles in the inflammatory reactions associated with exposure to ionizing or UV radiation. UVB induces expression of various cytokines in human keratinocytes (57–59). In particular the induction of release of IL-6 has been correlated with the formation of cyclobutane pyrimidine dimers (27). Similarly, various soluble factors are released following ionizing radiation. An increase in tumour necrosis factor α (TNFα) release has been observed in γ-irradiated human sarcoma cell lines (60) and in irradiated human T lymphocytes (61), but not in human endothelial cells (62). Interestingly, monocylic cell lines have been shown to produce TNFα after γ-irradiation (63), but this does not seem to be the case for freshly isolated human monocytes (64). Comparable observations have been made for ovarian tumour cells, where levels of TGFβ are reduced by γ-irradiation in freshly isolated cells. In contrast, with established cell lines, irradiation significantly enhanced TGFβ levels (34). These observations suggest that immortalization modulates cellular responses, including cytokine release, to ionizing radiation. We have compared the ability of both ionizing and UVB radiation to induce release of the inflammatory cytokine IL-6 in primary and immortalized keratinocytes. As with apoptosis, release of IL-6 is not enhanced by γ-radiation in primary cells but is induced efficiently by UVB. In the two immortalized keratinocyte cell lines IL-6 release was increased by both types of radiation. This distinction was also observed at the mRNA level. UVB radiation initiates release of the cytokine very rapidly in both NHEK and the keratinocyte cell lines. This is in contrast to the late onset of γ-radiation induced release of IL-6 in HeLa-KB and HaCaT cells. Thus there is a close similarity between the patterns of induction of cytokine release and apoptosis in these cultures.

We can speculate whether the results of our study of apoptosis may be relevant to the treatment of cancer. It is still unclear as to how apoptosis or mitotic death may contribute to the mode of death during tumour clearance following radiotherapy (65). The immortalized HeLa-KB and HaCaT cells can be considered to represent surrogate cancer cells [although HaCaT cells are not in fact tumorigenic (3,4)] and are clearly sensitive to apoptosis in comparison with the untransformed NHEK cells. This is particularly the case with the case with ionizing radiation and mitoxantrone, both successful treatments for cancer and both able to induce DSB in DNA, but unable to induce apoptosis in NHEK cells at the doses used here. In the in vivo situation this difference may be reflected as a sparing of untransformed keratinocytes and a targeting of the cancerous cell. Untransformed cells will undoubtedly be sterilized by the treatments but may not be triggered to undergo apoptosis and may thus maintain structural integrity until sloughed off by the normal processes of desquamation. Effective anticancer treatment may be associated with an absence of induction of apoptosis in normal tissue.

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