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Letter to the Editor

Apoptosis and efficient repair of DNA damage protect human keratinocytes against UVB

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Dear Editor,

Since the eighties it is well known that keratinocytes are more resistant to the lethal effects of UV light than fibroblasts, but the mechanism behind this phenomenon is still unknown. In the present study we investigated cell survival, apoptosis, cell cycle progression, UV photoproduct induction and repair, p53 gene response following UVB exposure in primary cultures of keratinocytes, and we compared the response with that of primary fibroblasts from the same skin biopsy. Keratinocytes are the primary target for UVB-induced human cutaneous malignancies, thus epidermal cells might have specific strategies to mantain genomic integrity. Nucleotide excision repair (NER) is a major defense mechanism against the deleterious effects of pyrimidine dimers (CPD) and 6-4 photoproducts (6-4 PP), the most biologically relevant damage induced by UV into DNA. The NER system has two distinct subpathways: global genome repair (GGR) that repairs lesions throughout the genome, and transcription-coupled repair (TCR) that operates on lesions in the transcribed strand of active genes (reviewed in Balajee and Bohr[1]). To efficiently repair damage, cells transiently arrest their growth at different points of the cell cycle (reviewed in Bartek and Lukas[2]). To limit the survival in the presence of irreparable DNA damage, cells die by apoptosis (reviewed in Kulms and Schwarz[3]). This phenomenon is evident in skin with the appearance of sunburn cells. In keratinocytes, UV-induced apoptosis is p53-dependent.[4]

The colony-forming ability of primary keratinocytes and fibroblasts from two independent skin biopsies was measured after UVB exposure (Figure 1a). Keratinocytes were more resistant to the lethal effects of UVB than the fibroblasts (D_{37} of 1000 J/m^2 and 500 J/m^2 for keratinocytes and fibroblasts, respectively). Cell death can occur via different mechanisms including apoptosis. Apoptosis was measured by TUNEL assay at different times after cell exposure to 1000 J/m^2 of UVB (Figure 1b). The number of apoptotic keratinocytes increased significantly at 24 and 72 h after UVB exposure whereas at the same dose fibroblasts were completely refractory to apoptosis. The activation of an apoptotic response by UVB in keratinocytes was confirmed by fluorimetric detection of caspase-3 (data not shown). Therefore, keratinocytes although more resistant to the lethal effects of UVB are more susceptible to UVB-induced apoptosis than fibroblasts.

The differential sensitivity to UVB of keratinocytes and fibroblasts might be because of differences in the level or repair of DNA damage in the two cell types. Fibroblasts and keratinocytes were exposed to 1000 J/m^2 of UVB and the amount of CPD and 6-4 PP was determined on the extracted DNA by ELISA using the specific antibodies. The yield of both DNA lesions was approximately 1.5-fold higher in fibroblasts than in keratinocytes but the ratio of CPD to 6-4 PP was similar in the two cell types (data not shown). In general, the loss of 6-4 PP was more rapid than that of CPD, as expected on the basis of their half-life (Figure 1c) (reviewed in Balajee and Bohr[1]). The repair rate of 6-4 PP was similar in both cell types. In contrast, CPD were repaired at a significant faster rate in keratinocytes than in fibroblasts. After 24 h irradiation, only 20% of the initial CPD were left in keratinocyte DNA whereas over 50% of the initial lesions remained in fibroblast DNA. The higher efficiency in repair of CPD by keratinocytes cannot be ascribed to the lower level of initial DNA damage since the repair kinetics in fibroblasts following a dose of 500 J/m^2 was similar to that reported after a dose of 1000 J/m^2 UVB (data not shown).

To address the question of whether cell cycle progression is differentially affected in the two cell types, cells were exposed to UVB and cell cycle position was determined 24 and 48 h after irradiation. A representative cell cycle distribution at 24 h post-irradiation is displayed in Figure 1d. In fibroblasts, at both UVB doses, a G_1–S phase arrest was observed whereas in keratinocytes the cell cycle distribution was substantially unaltered.

The level of the stress response protein p53 was determined after irradiation. Both fibroblasts and keratinocytes responded to UVB damage (1000 J/m^2) with stabilization of the p53 protein (Figure 1e). However, while in fibroblasts p53 displayed a significant increase at 12 h after irradiation and continued to accumulate up to 24 h, in keratinocytes p53 level reached a peak at 6 h and then drastically decreased to background at 12 h. Moreover, higher levels of UVB-induced p53 protein were observed in fibroblasts than in keratinocytes. In keratinocytes, a rapid but transitory p53 response to UVB was observed also after 2000 J/m^2 (data not shown).

From this study, apoptosis and an efficient DNA repair machinery for UV photoproducts emerged as the major defense mechanisms of keratinocytes against the deleterious effects of UVB. Keratinocytes undergo apoptosis at UVB doses that are ineffective in fibroblasts. It has been proposed that the stalling of the transcription machinery at CPD leads to activation of p53, thus initiating apoptosis.[5] This model is strongly supported by the finding that in TCR-defective fibroblasts, derived from patients with Cockayne syndrome, p53 and apoptosis are induced at UVC doses that are

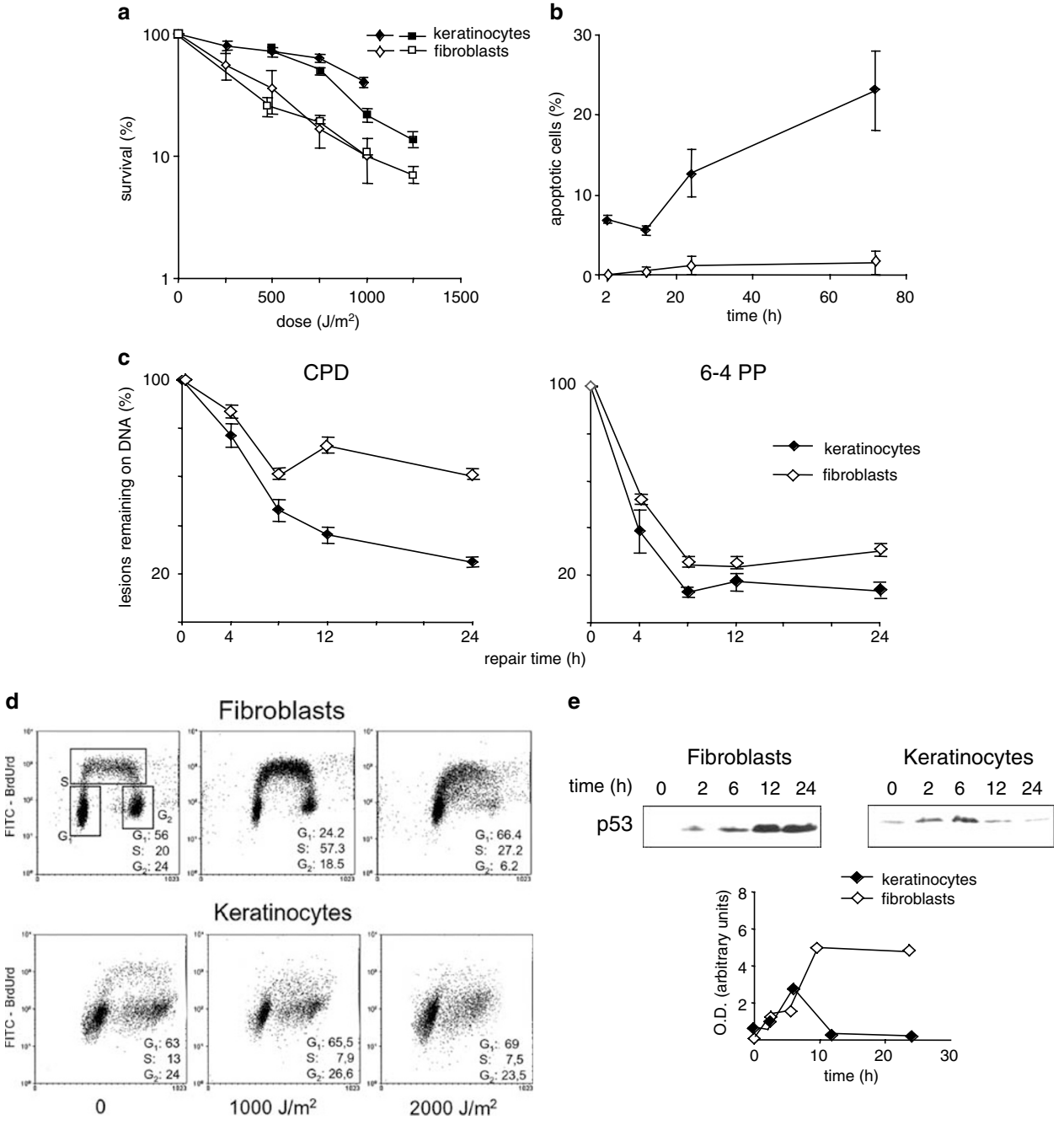

Figure 1 (**a**) Cell survival after UVB exposure. Data for fibroblasts and keratinocytes cultures from two baby foreskin biopsies are displayed. Keratinocytes were cultivated on a feeder layer of lethally irradiated 3T3-J2 fibroblasts (a gift from H. Green, Harvard Medical School, Boston, MA), as previously described.[14] The colony-forming ability of proliferating cells was measured following irradiation with FST12 sunlamps. The number of colonies in irradiated samples was expressed as a percentage of that in unirradiated samples. The data are the mean of at least two independent experiments each performed in triplicate. The bars indicate the standard errors. (**b**) UVB-induced apoptosis. Apoptosis was measured by the TUNEL assay (*in situ* cell death detection kit, Boehringer Mannheim) at different times after cell exposure to 1000 J/m^2 of UVB. At least 100 cells were scored per experimental point. (**c**) Repair of UV photoproducts. Cells were exposed to 1000 J/m^2 of UVB and allowed to repair for different periods of time. DNA was extracted with the Qiagen kit (Genenco). The level of photoproducts was measured in microtiter plates, coated with protamine sulphate (30 ng for CPDs and 200 ng for 6-4PP) using TDM-2 and 6-4M2 monoclonal antibodies in a standard ELISA technique as previously described.[15] No cell duplication occurred during the 24 h post-irradiation time in either cell type, thus no correction was made for cell damage dilution. The data are the mean of four independent experiments. The bars indicate the standard errors. (**d**) Cell cycle response to UVB. Fibroblasts and keratinocytes were irradiated with a single dose of 1000 or 2000 J/m^2 and fixed 24 h after exposure. At 30 min before processing, cells were labeled with 45 M BrdUrd. Bivariate dot blots show the distribution of the green fluorescence of the FITC anti-BrdUrd staining (DNA synthesis; *Y*-axis) versus the red fluorescence of the propidium iodide staining (DNA content; *X*-axis). Experiments were repeated at least two times, 10 000 events were recorded for each sample and data analysis was performed by using Cell-Quest software (Becton Dickinson). (**e**) Induction of p53 by UVB. Protein lysates were obtained from unirradiated keratinocytes and fibroblasts and after different times post-UVB exposure to 1000 J/m^2. Top: Western blot analysis. The samples (100 μg) were probed with anti-p53 antibodies (Santa Cruz Biotechnology). To normalize the expression level of p53, the samples were also probed with anti-β actin (for fibroblasts) and anti-14-3-3 ξ (for keratinocytes) antibodies (Santa Cruz Biotechnology). Detection was by ECL (Amersham). Bottom: densitometric analysis of protein bands by using Gel Doc 2002 analysis program (Biorad). The values were normalized for expression of the housekeeping genes as specified above

significantly lower than those required for normal cells.[6–8] Besides the transcription blockage, the state of the proliferation of the cells may be involved in the induction of apoptosis. In p53-defective rodent cells UVC-induced apoptosis requires replication-dependent formation of double-strand breaks.[9] It is tempting to speculate that in keratinocytes the lack of cell cycle arrest in response to UVB might increase the likelihood of replication through unrepaired DNA lesions, thus leading to apoptotic death at doses that in fibroblasts would block the cells in G_1/S thus allowing repair to occur. Alternatively or additionally, a receptor-initiated pathway might contribute to UVB-induced apoptosis in keratinocytes (reviewed in Zhuang *et al.*[10]). Apoptosis, however, involves a small amount of irradiated keratinocytes. DNA repair is the additional surveillance mechanism that ensures the transmission of correct genetic material. The level of UV photoproducts was slightly but consistently lower (1.5-fold) in keratinocytes as compared with fibroblasts following exposure to the same UV doses as previously reported.[11] It is well known that 6-4 PP are removed rapidly from DNA, predominantly by GGR, whereas CPD are repaired very slowly by GGR and more efficiently from the transcribed strand of expressed genes by TCR (reviewed in Ura and Hayes[12]). The repair kinetics of CPD in fibroblasts was biphasic with 20–30% of lesions repaired in the first 12 h and an additional 10–20% in the following 12 h. In contrast, only 20% residual CPD were detected on keratinocyte DNA after 24 h post-irradiation (when 60% of CPD are still present on fibroblast DNA). In keratinocytes, the more efficient removal of CPD from bulk DNA is likely to be ascribed to GGR (reviewed in Balajee and Bohr[1]). However, we cannot exclude that a more efficient TCR might also contribute to the accelerated repair of CPD, thus providing these epidermal cells of a highly effective protection mechanism for genome integrity.

Under our irradiation regimen, keratinocytes undergo apoptosis but the cell cycle progression is not significantly affected. In agreement with this finding, the levels of p53 after irradiation are significantly lower in keratinocytes than in fibroblasts that experience an abrupt inhibition of G_1 to S phase progression. A different cell cycle arrest profile in response to DNA damage in keratinocytes as compared with fibroblasts has been previously reported after exposure to γ-irradiation and adriamycin.[13] Also in this case, keratinocytes presented an attenuated G_1 arrest whereas fibroblasts arrested in G_1. These observations indicate that the cell cycle response to DNA damage may vary depending on the cell type.

The striking difference in the kinetics of p53 induction that we have observed in the two cell types deserves some comment. Several lines of evidence indicate that the level of CPD in the transcribed strand of active genes determines the cellular level of p53 by providing the signal for its induction. It has been recently shown that almost all the signal for p53 induction after UVB in mice involves DNA photoproducts in transcribed genes.[5] The lower level of induction and the rapid decrease of p53 in keratinocytes as compared with fibroblasts is consistent with lower levels of DNA damage and/or more efficient repair in these specialized epidermal cells.

Most information on UV response has been obtained from studies on dermal fibroblasts. Our results demonstrate that there is a significant diversity in the biological response to UVB of keratinocytes and fibroblasts. This is important especially with respect to human cancer since epidermal cells are the target for UVB-induced skin carcinogenesis.

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