Peculiarities of Cell Cycle Kinetics in Normal Keratinocytes and Melanocytes after Exposure to UV-B Radiation

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We studied changes in cell cycle and apoptosis in normal keratinocytes and melanocytes after exposure to UV-B radiation. UV-B radiation modulated proliferation rate in the studied cell and produced an apoptosis-modulating effect in epidermal melanocytes.

Key Words: *apoptosis; cell proliferation: UV radiation*

Ultraviolet radiation is one of the main factors inducing skin malignancies. Cell cycle and apoptosis dysregulation play a key role in the progress of oncological disease. Epidermal melanocytes and keratinocytes are characterized by different sensitivity to UV radiation, which probably provides the basis for the mechanisms underlying the development of tumors originating from these cell types.

Here we studied the effects of different doses of UV-B radiation on cell proliferation and caspase-3-mediated apoptosis in normal keratinocytes and melanocytes.

METHODS

Normal keratinocytes (NHEK cell line, Clonetics) were cultured in KGM-2 medium (Clonetics) supplemented with epinephrine, epidermal growth factor, insulin, transferrin, gentamicin, amphotericin B, hydrocortisone, and bovine pituitary extract [3]. Normal melanocytes (HEM, Cascade biologics) were cultured in Medium 254 (Cascade biologics) supplemented with bovine pituitary extract, fetal bovine serum, bovine insulin, bovine transferring, basic fibroblast growth factor, hydrocortisone, heparin, phorbol myristate acetate [11]. The cell cultures were grown at 37°C and 5% CO_2. After attaining 70-80% confluence, the cells were trypsinized and subcultured in Petri dishes. After 24 h, the cells were exposed to UV light with maximum wavelength of 301 nm (UVM-57 model, San-Gabriel equipped with Kodacel filter, Eastman Kodak Co.). The 30-, 60-, 90-, and 120-sec exposures corresponded to doses of 120, 240, 360, and 480 J/m^2, respectively.

After 24 h, the cells were trypsinized and lyzed. The intensity of cell proliferation was evaluated by PCNA level [9], caspase-3 was used as apoptosis marker [8]. Immonoblotting procedure was performed in accordance with standard protocol using antibodies to PCNA (proliferating cells nuclear antigen; 1:200; Santa Cruz Biotechnology Inc.) and caspase-3 (1:1000; Biomol International). Incubation with secondary antibodies conjugated with horseradish peroxidase (dilution 1:5000, Transduction Laboratories) was performed for 1 h; visualization was conducted using ECL (Amersham Biosciences). Membranes were reincubated with antibodies to glyceraldehyde phosphate dehydrogenase (GADPH; 1:10,000; Trevigen Inc.) in order to confirm identical protein content in all specimens during electrophoresis. The expression of PCNA and caspase-3 in each sample was measured using Kodak 1D 3.6 software as the ratio of fluorescence intensity of PCNA or caspase-3 band to the intensity of the GADPH band [6]. The results were reproduced 5 times.

Irradiation of skin cells was conducted using an UV source (λ=302 nm). This type of irradiation

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correspond to UV-B band and was used in the experiment, because UV-B radiation produces maximum effect on epidermal cells and modulates functional activity of keratinocytes, melanocytes, and Langerhans cells [3]. Moreover, carcinogenic effect of UV-B radiation was established [4].

The results obtained before and after treatment were statistically analyzed using Wilcoxon *W* test [1].

RESULTS

Significant decrease in the intensity of keratinocyte proliferation evaluated by PCNA expression was observed only after exposure to UV at 480 J/m^2. Caspase-3 level did not differ from the control level under these conditions (Table 1). In normal melanocytes, UV irradiation produced visually detectable changes: the cell size and length of processes decreased (Figs. 1, 2, and 3). PCNA level decreased; changes in the content of caspase-3 were observed (Table 2).

In normal keratinocytes, the level of caspase-3 remained unchanged which indicates that exposure to UV irradiation in the selected doses did not cause activation of caspase-3-dependent apoptosis. In melanocytes, we observed inhibition of proliferation and changes in caspase-3 expression with significant increase in enzyme content after exposure at 240 J/m^2.

Keratinocytes are intensively proliferating cells [8]. Moreover, UV exposure induces apoptotic death of keratinocytes with disintegration of mitochondrial membranes, cytochrome *C* release, and caspase activation [10]. Our results indicate that UV irradiation in the maximum dose of 480 J/m^2 is insufficient for induction of apoptosis in keratinocytes.

Mechanisms of melanocyte apoptosis and proliferation are less studied. According to some re-

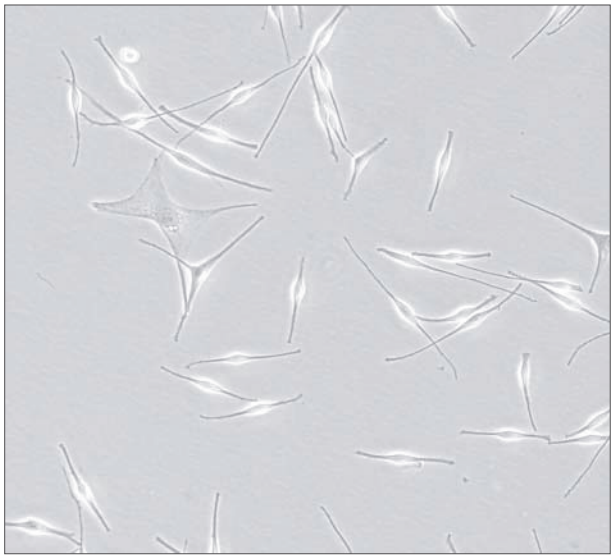

Fig. 1. Normal melanocytes before exposure to UV irradiation.

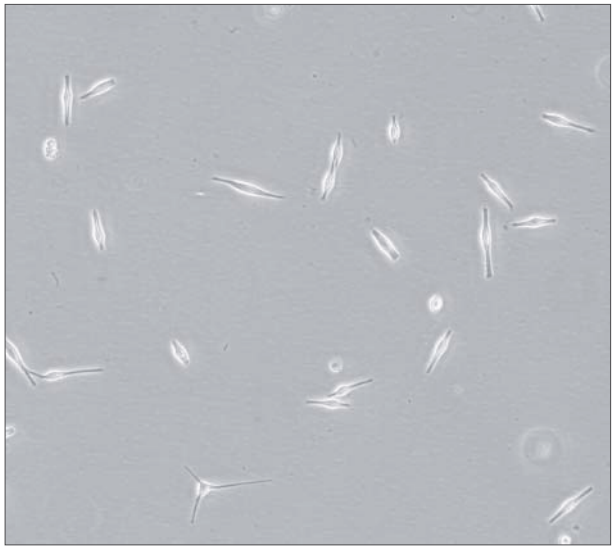

Fig. 2. Normal melanocytes 24 h after exposure to UV irradiation in a dose of 480 J/m^2.

TABLE 1. Expression of PCNA and Caspase-3 in Normal Keratinocytes after Exposure to UV Irradiation in Doses of 120-480 J/m^2

Index	Control (*n*=5)	120 J/m^2 (*n*=5)	240 J/m^2 (*n*=5)	360 J/m^2 (*n*=5)	480 J/m^2 (*n*=5)
PCNA/GADPH, rel. units	1.41	1.13	2.21	1.86	1.02*
Caspase-3/GADPH, rel. units	0.06	0.09	0.22	0.08	0.05

Note. Here and in Table 2: *α=0.01 compared to the control.

TABLE 2. Expression of PCNA and Caspase-3 in Normal Melanocytes after Exposure to UV Irradiation in Doses of 120-480 J/m^2

Index	Control (*n*=5)	120 J/m^2 (*n*=5)	240 J/m^2 (*n*=5)	360 J/m^2 (*n*=5)	480 J/m^2 (*n*=5)
PCNA/GADPH, rel. units	1.94	0.73*	0.28*	0.18*	0.11*
Caspase-3/GADPH, rel. units	0.09	0.04*	0.1*	0.05*	0.04*

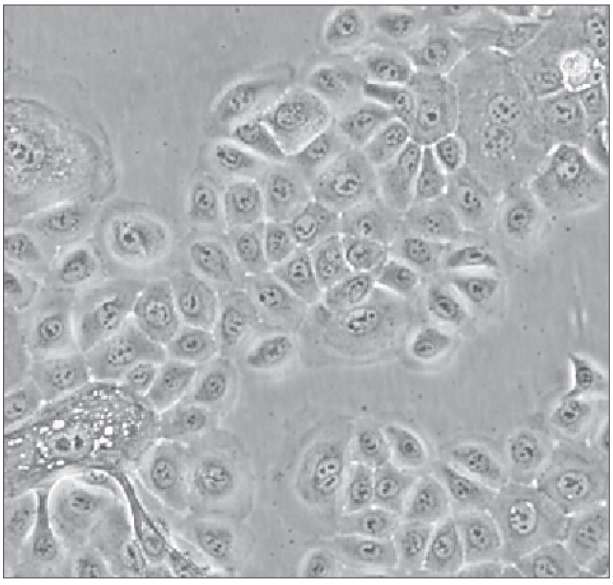

Fig. 3. Normal keratinocytes before exposure to UV irradiation.

ports, UV irradiation activates melanocyte proliferation [5]. The role of caspase-3 in melanocyte apoptosis remains poorly understood. Melanocyte resistance to apoptosis-inducing stimuli can be explained by high level of expression of antiapoptotic protein Bcl-2 [2]. In our experiment we detected significant changes in caspase-3 level in melanocytes and a decrease in their proliferative activity, which probably attests to the realization of programmed cell death in melanocytes after exposure to UV-B in doses of 120-480 J/m^2.

The study demonstrates that UV-B in doses of 120-480 J/m^2 inhibits proliferation of melanocytes and keratinocytes. UV-B in the specified doses produces no apoptosis-modulating effect on keratinocytes, but changed the level of caspase-3 in melanocytes. Mechanisms of induction and realization of apoptosis differ in epidermal keratinocytes and melanocytes. The study demonstrates the possibility of inducing apoptosis in melanocytes by UV-B in doses up to 480 J/m^2.

The study of the effects of UV irradiation on apoptosis and intensity of cell proliferation in normal and tumor epidermal cells is required for better understanding of the molecular mechanisms underlying the development of skin malignancies. Specific features of cell cycle kinetics in these cell types can explain the differences in the biological behavior of skin tumors, basal cell carcinoma and melanoma.

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