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The Signaling of UV-induced Apoptosis in Melanocytes

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Ultraviolet B (UVB) radiation may activate or deteriorate cultured human epidermal melanocytes, depending on the doses and culture conditions. In this study, we examined whether apoptosis of melanocytes can be induced by physiologic doses of UVB irradiation. PI staining for DNA condensation and flow cytometric analyses demonstrated the apoptotic cell death of melanocytes after UVB irradiation. The level of p53 and Bax revealed a dose-dependent increase with increasing dose of UVB, but the level of Bcl-2 remained unchanged. Confocal microscopic examination showed that Bax moved from a diffuse to a punctate distribution after UVB irradiation. However, there were no changes in the pattern of Bcl-2. We next examined the downstream targets of apoptosis. Our results showed that a precursor form of caspase-3 disappeared with increasing doses of UVB. We also observed cleavage of poly(ADP-ribose) polymerase (PARP) after UVB irradiation. In addition, UVB irradiation resulted in a remarkable activation of c-Jun N-terminal kinase (JNK). These results indicate that UVB may induce apoptosis via JNK activation in human melanocytes.

Key words : apoptosis, UVB, melanocytes

INTRODUCTION

Apoptosis or programmed cell death is a genetically regulated, cellular suicide mechanism that plays a crucial role in the development as well as in the maintenance of homeostasis. Ultraviolet (UV) light is one of the injurious environmental agents that are known to lead to apoptosis of cells. Vitiligo, characterized by loss of melanocytes, has been considered an autoimmune disorder. However, various factors including sunburn are implicated as precipitating factors in about 25% of vitiligo patients. These observations suggest that ultraviolet may induce oxidative stress in the skin and cause cell death of melanocytes. However, studies on UVB-induced apoptosis of melanocytes are still lacking and there are some discrepancies between researchers. Therefore, we have tried to evaluate the characteristics of UVB-induced apoptosis of human melanocytes.

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Table 1. The percentages of cells in the individual quadrants by flow cytometric analysis of UVB-irradiated melanocytes. (upper left panel: necrotic cells; upper right panel: late stage apoptosis or necrotic cells; lower left panel: viable cells; lower right panel: early apoptosis)

Serum	UVB	UL(%)	UR(%)	LL(%)	LR(%)
(+)	0 mJ/cm^2	24.68	1.56	69.90	3.86
	15 mJ/cm^2	15.11	4.98	71.63	8.28
	30 mJ/cm^2	9.39	4.98	73.08	12.55
(-)	0 mJ/cm^2	9.66	6.14	56.52	27.68
	15 mJ/cm^2	8.78	6.16	56.36	28.70
	30 mJ/cm^2	8.14	9.32	46.36	36.18

MATERIALS AND METHODS

Cell culture. Normal human melanocytes were isolated from the foreskins and maintained at 37℃ in 5% CO_2 with a growth medium as previously described [1].

MTT assay. Cell viability was determined by the MTT assay.

UVB irradiation. UVB was irradiated as previously described [2]. After UVB irradiation, the cells were placed immediately in the same medium.

Nuclear staining. Nuclear staining was performed as previously described [2]. Morphology of nuclei was examined using a fluorescent microscope.

Flow cytometric analysis. Apoptosis was analyzed using a FACS Calibur (Becton-Dickinson, San Jose, CA) as previously described [2].

Western blot analysis Western blot analysis was performed as previously described [2].

Confocal microscopic examination. Cells were observed with a Bio-Rad confocal microscope (MRC 1024, Hercules, CA) as previously described [2].

RESULTS

UVB irradiation induces apoptosis in melanocytes. Cultured normal human melanocytes showed no definite nuclear condensation 24hrs after UVB irradiation. FBS was then withdrawn two days prior to UVB irradiation. After UVB irradiation, cells were stained with PI. Upon exposure to UVB, the number of PI stained melanocytes increased in a dose-dependent manner. Most of the PI-positive cells displayed condensed, homeogenous, and fragmented nuclei (data not shown). Flow cytometric analysis was performed and the UVB irradiated cells were categorized into three groups; healthy (annexin V^-/PI^-), early apoptotic (annexin V^+/PI^-), and late apoptotic or necrotic (annexin V^+/PI^+). Compared to complete media condition, number of apoptotic cells was much higher in serum deprived conditions (Table 1).

UVB increased p53 and Bax, but did not affect Bcl-2. Twenty four hours after UVB irradiation, the level of p53 gradually increased with increasing doses of UVB,

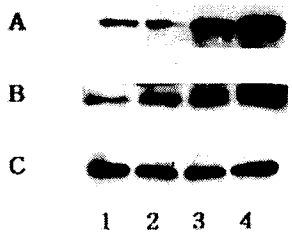

Figure 1. Western blot analysis of p53, Bax, and Bcl-2 after UVB irradiation (A: p53, B: Bax, C: Bcl-2, 1: 0 mJ/cm^2, 2: 10 mJ/cm^2, 3: 20 mJ/cm^2, 4: 30 mJ/cm^2).

Figure 2. Confocal microscopic examination of Bax in UVB irradiated melanocytes. Before UVB irrdiation (A). After UVB irradiation, a punctate distribution of Bax was observed (B). Twenty four hours after UVB irradiation, Bax was localized to the peri-nuclear area (C).

reaching a maximum level at 30 mJ/cm^2 (Fig.1). The level of Bax protein also increased with increasing doses of UVB up to 30 mJ/cm^2. However, the Bcl-2 level did not change throughout the range of 0-30 mJ/cm^2.

Changes of Bax by confocal microscopic examination. Immediately after UVB irradiation, the staining intensity of Bax in the melanocytes increased rapidly. By decreasing the fluorescence staining intensity, it was found that Bax showed a punctate pattern. Twenty four hours after UVB irradiation, Bax moved to the peri-nuclear area (Fig.2). However, no change was observed in the distribution of Bcl-2.

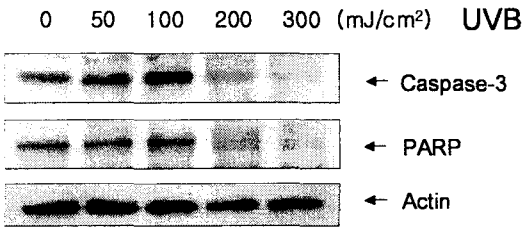

Figure 3. Western blot analysis of caspase-3 and PARP after UVB irradiation.

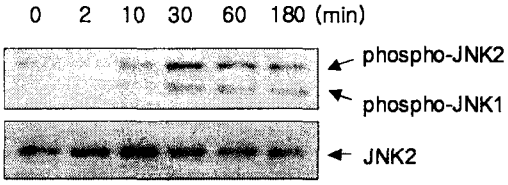

Figure 4. Western blot analysis of JNK phosphorylation after 200 mJ/cm^2 UVB irradiation.

UVB activated caspase-3 and cleaved PARP. We also examined the downstream targets of apoptosis. Our results showed that a precursor form of caspase-3 disappeared with increasing doses of UVB, indicating that UVB irradiation activated caspase-3. The active form of caspase-3 cleaves several other proteins such as PARP. We also observed cleavage of PARP after UVB irradiation (Fig.3).

UVB led to the activation of JNK. To investigate the effects of UVB irradiation on the stress-regulated kinase regulation in melanocytes, we next examined the change of JNK activation. A dose of 200 mJ/cm^2 UVB irradiation resulted in a remarkable and sustained activation of JNK. These results indicate that UVB may induce apoptosis via JNK activation in human melanocytes.

DISCUSSION

Zhai et al reported that physiologic doses (5-25 mJ/cm^2) of UVB induce characteristic apoptotic death in melanocytes when they are deprived of TPA, FBS and hydrocortisone [3]. Similarly, our experiments indicated that UVB and serum deprivation could induce apoptotic death in melanocytes. It is widely believed that melanocytes have an innate resistance to apoptosis induced by various injuries including UV irradiation, and that the high level of constitutional Bcl-2 in melanocytes makes a significant contribution to this resistance. In our experiment, the basal level of Bcl-2 was high and remained unaffected in cultured normal human melanocytes after UVB irradiation. Thus, melanocytes appear to be protected from UVB by high levels of Bcl-2 *in vitro*. We also examined the staining pattern of Bcl-2 and Bax in UVB irradiated melanocytes by confocal microscopic examination, and did not observe a change in the distribution pattern of Bcl-2 in UVB irradiated melanocytes. It is in agreement with the previous report that there were no changes in the pattern of Bcl-2 in apoptotic Cos-7 cells. However, Bax moved from a diffuse to a punctate pattern immediately after UVB irradiation. Furthermore, there was intense staining of Bax compared to control cells. Twenty four hours after UVB irradiation, Bax was localized to the peri-nuclear area. It has also been reported that Bax undergoes a shift from a diffuse to a punctate distribution pattern during apoptosis in Cos-7 cells. A punctate localization of Bax was regarded as being due to the collection of Bax at mitochondria.

In our study, UVB irradiation induced apoptosis in cultured melanocytes and the elevation of p53. Bax was up-regulated and moved to the peri-nuclear area after UVB irradiation, but Bcl-2 was not affected at 24h after UVB irradiation. Previously, it was reported that Bcl-2 is significantly reduced in human melanocytes at 48 and 72 hours after UVB exposure. Therefore, one might conclude that the increase and redistribtuion of Bax is an early event that initiates the apoptotic response to UVB, and it is then followed by a reduction in Bcl-2.

It has also been reported that a family of cysteine proteases called caspases is involved in apoptotic process. The active form of caspase-3 cleaves several other apoptotic proteins such as PARP. Our experiments showed that UVB activated caspase-3 and cleaved PARP. Therefore, the UVB-induced apoptotic death of human melanocytes may be induced by the cleavage of PARP and mediated by the activation of caspase-3. The activation of JNK by UVB indicated JNK might be involved in apoptosis of human melanocytes.

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