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Antiapoptotic Effects Induced by Different Wavelengths of Ultraviolet Light

Yuko Ibuki* and Rensuke Goto
Graduate School of Nutritional and Environmental Sciences, University of Shizuoka
52-1, Yada, Shizuoka-shi 422-8526, Japan

Cells receive signals for survival as well as death, and the balance between the two ultimately determines the fate of the cells. UV-triggered apoptotic signaling has been well documented, whereas UV-induced survival effects have received little attention. We have reported previously that UVB irradiation prevented apoptosis, which was partly dependent on activation of the phosphatidylinositol 3-kinase (PI3-kinase)/ Akt pathway. In this study, anti-apoptotic effects of UV with different wavelength ranges, UVA, UVB and UVC, were examined. NIH3T3 cells showed apoptotic cell death by detachment from the extracellular matrix under serum-free conditions, which was prevented by all wavelengths. However, the effect of UVA was less than those of UVB and UVC. Reduction of mitochondrial transmembrane potential and activation of caspase-9 and –3 were suppressed by all three wavelengths of UV, showing wavelength-dependent effects as mentioned above. The PI3-kinase inhibitor wortmannin partially inhibited the UVB and UVC-induced suppression of apoptosis, but not the inhibitory effect of UVA. The Akt phosphorylation by UVB and UVC was completely inhibited by addition of wortmannin, but that by UVA was not. P38 MAP kinase inhibitor SB203580 partially inhibited the UVB and UVC-induced suppression of apoptosis and Akt phosphorylation, and completely inhibited UVA-induced those. These results suggested the existence of two different survival pathways leading to suppression of apoptosis, one for UVA that is independent of the PI3-kinase/Akt pathway and dependent on p38 MAP kinase, and the other for UVB and UVC that is dependent on both pathways.

Key words : apoptosis, ultraviolet light, mitochondria, PI3-kinase, Akt, p38

INTRODUCTION

Apoptosis is the most important cell death system to avoid the cancer caused by UV-induced DNA damage. Many investigations have shown that the induction of apoptosis by UV irradiation is initiated by DNA damage and is dependent on p53 up-regulation. Cells receive signals for survival as well as death, and the balance between the two ultimately determines the fate of the cells. UV-triggered apoptotic signaling has been well documented as described above; however, much less is known about the UV-induced survival signals. We found that UVB irradiation suppressed apoptosis in cells detached from the extracellular matrix (ECM) under serum depletion via activation of the phosphatidylinositol 3-kinase (PI3-kinase) /Akt survival pathway [1]. This system proved the survival signals by UVB irradiation clearly *in vitro*. Recently, Wan et al. also showed UVB-induced activation of PI3-kinase/Akt pathway via EGF receptors in human skin *in vivo*, suggesting the possibility of the promotion of skin cancer [2]. The fact that cells leading to apoptosis are survived by UVB irradiation suggests an increase of neoplastic risk by UVB irradiation because mutated DNA-containing cells survived and passed the mutated DNA to daughter cells. Therefore,

* To whom correspondence should be addressed.
E-mail: ibuki @u-shizuoka-ken.ac.jp

further studies in other wavelengths, especially in UVA region, were required from the views of usual UVA-exposure and cancer promotion. In this study, we examined the differences of anti-apoptotic effects between UVA, UVB and UVC. The goal of this study was to elucidate the wavelength-dependent differences of survival signal pathways leading to anti-apoptosis.

MATERIALS AND METHODS

Apoptosis induction and UV irradiation

NIH3T3 cells cultured in serum-free DMEM were trypsinized and plated in cell suspension dishes previously coated with heat-denatured BSA (2mg/ml) at 37°C for 1h. The cells were immediately irradiated at room temperature. Wavelength characteristics of the UV lamps used were as follows. UVA lamp, spectral output of 3% in the UVB (<320nm), 17% UVA2 (320~340nm) and 74% UVA1 region (340~400nm) with an emission wavelength peak of 365 nm. UVB lamp, spectral output of 1% in the UVC (<280nm), 58% UVB, 26% UVA2 and 15% UVA1 region (340~400nm) with an emission wavelength peak of 312 nm. UVC lamp, output of 98% at a wavelength of 254nm was used.

Apoptosis induction was determined by morphological nuclear changes (Hoechst33342 staining) and appearance of subdiploid apoptotic nuclei (PI staining).

Determination of mitochondrial transmembrane potential ($\Delta\Psi$) and caspase activity

$\Delta\Psi$ was detected by staining with rhodamine 123. Caspase activity was measured by the direct assay of caspase enzyme activity in cell lysates as previously described [1].

Immunoblotting analysis for Akt

Following incubation for 15 min after UV irradiation, cells were lysed in SDS sample buffer. The lysates were separated by SDS-PAGE followed by electroblotting onto PVDF membranes.

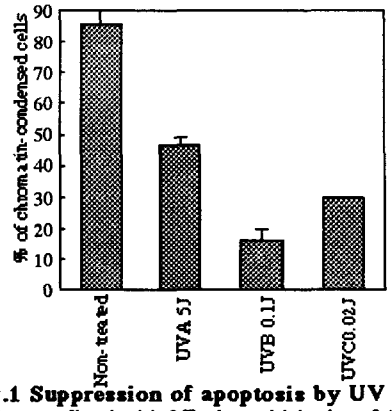

Fig.1 Suppression of apoptosis by UV irradiation
Cells were fixed with 2% glutaraldehyde at 24h after UV irradiation and stained with 1mM Hoechst 33342 for 10 min. Aliquots were placed on glass slides, and 200 cells per slide were scored microscopically in quadruplicate.

The membranes were incubated with phosphospecific (Ser473) anti-Akt IgG or anti-Akt IgG followed by HRP-conjugated anti-rabbit IgG. Signals were detected with the ECL system.

RESULTS AND DISCUSSION

Inhibition of apoptosis by all wavelengths of UV

All wavelengths of UV inhibited cell death induced by detachment from the ECM under serum-free conditions. The effect of UVA was less than 50 % of that of UVB and UVC irradiation. The most significant effect of UVA was observed at a dose of 5J/cm^2. UVB dose of 0.1J/cm^2 and UVC doses of 0.02~0.05J/cm^2 significantly inhibited cell death, respectively. Irradiation by all three wavelengths prevented the induction of chromatin condensation (Fig. 1) and the appearance of subdiploid apoptotic nuclei. These results suggested that UV irradiation suppressed apoptosis wavelength-independently, although the effect of UVA was less than those of UVB and UVC.

Inhibition of apoptosis upstream of the caspase cascade

To examine the inhibition of apoptosis by the three wavelengths of UV upstream of the nuclear signals, $\Delta\Psi$ was determined using staining with rhodamine 123. The peak of $\Delta\Psi$low was almost completely abolished by UVA irradiation

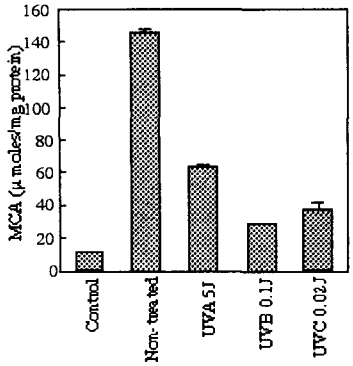

Fig.2 Inhibition of caspase-3 activation by UV irradiation
Following incubation for 6h after UV irradiation, cells were lysed and caspase-3 enzymatic activity in the lysate was determined using Ac-DEVD-MCA. Control, cultured in the presence of CS without irradiation; nontreated, cultured in the absence of CS without irradiation.

and completely abolished by UVB and UVC irradiation. Furthermore, caspase activities were determined using a synthetic fluorogenic substrate. Caspase-3 activity was markedly suppressed by UV irradiation (Fig.2). Degrees of suppression by UVB and UVC were higher than that of UVA. Initiator caspases, caspase-8 and-9, were slightly activated by detachment from the ECM under serum-free conditions. Suppression of caspase-9 activity by UVA was slightly less than that of UVB and UVC, which was in agreement with those of caspase-3 and reduction of $\Delta\Psi$. On the other hand, shorter wavelengths were associated with greater inhibitory effects on caspase-8 activities.

Activation of PI3-kinase/Akt and p38 MAP kinase pathway

UVB and UVC-induced anti-apoptotic effects were partially inhibited by PI3-kinase inhibitor wortmannin, whereas UVA-induced effect was not. On the other hand, p38 MAP kinase inhibitor SB203580 completely inhibited UVA-induced anti-apoptotic effect and partially inhibited UVB- and UVC-induced effect. Fig. 3A shows expression of Akt and phosphorylated Akt 15min after UV irradiation. Wortmannin completely inhibited the Akt phosphrylation by UVB and UVC, whereas that by UVA was observed even in the presence of wortmannin. Furthermore, SB203580 partially inhibited the Akt phosphrylation by UVB and UVC, whereas that by UVA was completely inhibited in the presence of SB203580.

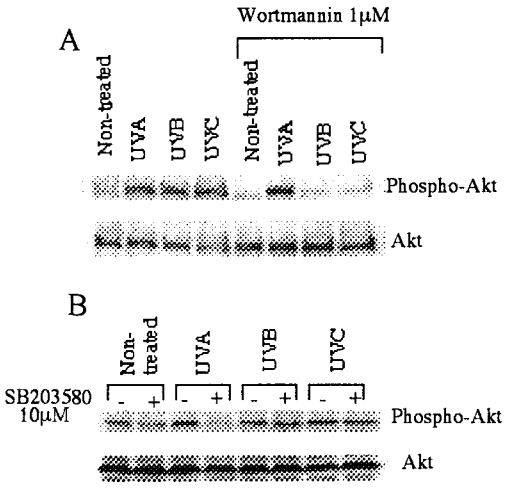

Fig.3 Phosphorylation of Akt after UV irradiation
Cells were treated with wortmannin (1μM) (A) or SB203580 (10μM) (B) for 1h before UV irradiation and cultured for 15 min. They were lysed in SDS sample buffer and subjected to SDS-PAGE followed by Western blotting with phosphospecific anti-Akt IgG and anti-Akt IgG.

These findings indicated the existence of two different survival pathways leading to suppression of apoptosis, one for UVA that is dependent on the p38 MAP kinase and independent of the PI3-kinase/Akt pathway and the other for UVB and UVC that is dependent on both pathways. Apoptosis is an important system to reduce neoplastic risk. The present study suggested that all wavelengths of UV could enhance the appearance of mutated cells by the inhibition of apoptosis through activation of survival signals. Further examinations of the survival signal cascades in this system may provide us a new insight for cancer promotion.

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