Similarity of Intracellular Signaling Toward Apoptosis Following UVB and UVC Irradiation

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UV irradiation activates various intracellular signaling pathways causing cell death in a DNA damage-dependent and an independent manner. As DNA photoproducts, major forms of DNA damage, are maximally formed by UV light at 260-nm, short wavelength UV (UVC) is more harmful than middle wavelength UV (UVB). However, the differences or similarities in responses of DNA damage-independent intracellular signaling molecules to UVB and UVC are not elucidated. We examined activation of signaling molecules towards apoptosis in normal human fibroblastic cells after irradiation with UVB or UVC at a dose generating the equal amount of DNA photoproducts. Both UVB and UVC induced transient phosphorylation of ERK and sustained phosphorylation of p38. Phosphorylation of p53 at Ser15 and at Ser392 residues were also observed, which were inhibited by a phosphoinositide 3-kinase inhibitor ,wortmannin. In contrast, an antioxidant N-acetyl-cysteine and a p38 inhibitor SB203580 suppressed only Ser392 phosphorylation, suggesting that UV-induced oxidative stress and p38 activation were involved in the phosphorylation of this site. The apoptic signals such as mitochondrial cytochrome C release and annexin V binding were then observed. Overall, no difference was found in chronological responses of p53, MAPK, and apoptosis between UVB-irradiated and UVC-irradiated cells. These results suggested that DNA damage-independent intracellular signaling molecules similarly responded to UVB and UVC when the equal level of DNA photoproducts were generated.

Key words : UVB, UVC, MAPK, p53, apoptosis

INTRODUCTION

UV-irradiation causes DNA damage which triggers several signaling pathways including p53 [1]. The activated p53 then induces apoptosis or cell cycle arrest leading to replicative cell death. On the other hand, there is mounting evidence that UV is able to elicit cellular responses through several intracellular molecules that are apparently not involved in damaging the DNA [2]. Our

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previous investigation demonstrated that the extent of UV-induced oxygen stress alters the lethal effect of UV on cultured cells and that signaling molecules such as MAP kinases were involved in this process [3]. Therefore, the overall responses of DNA damage-dependent and independent pathways may determine cellular outcome after UV-irradiation.

As DNA photoproducts, major forms of DNA damage, are maximally formed by UV light at 260-nm, short wavelength UV (UVC) is more harmful than middle wavelength UV (UVB). However, the differences or similarities in contribution of DNA damage-independent pathways to the lethal effect of UV at different wavelength are not elucidated. In this study, we examined responses of several signaling molecules to UVB or UVC at doses generating the equivalent amount of DNA photoproducts.

MATERIALS AND METHODS

Normal human embryonic fibroblast-like cells (HE49) were used in this study. These cells were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, at 37°C in a humidified atmosphere of 5% CO2-95% air. All the experiments were performed using the cells between seven and fifteen passages. UVB or UVC irradiation was performed using the 312-nm or 254-nm source with a dose rate of $100J/m^2/sec$ or $1J/m^2/sec$, respectively. Cyclobutane pyrimidine dimers (CPD) and (6-4)photoproducts (64PP) were quantitated by ELISA [4]. p53, ERK, JNK, p38 and their phosphorylated forms were detected by Western blot analysis. Clonogenic cell death was determined by colony formation. Apoptic cell death was examined by Western blot analysis for BAX protein and mitochondrial cytochrome C release, and Annexin V staining.

RESULTS AND DISCUSSION

Both UVB and UVC generated CPD and 64PP in a dose-dependent manner. UVB at $3200J/m^2$ and UVC at $16J/m^2$ formed an equal amount of CPD as well as 64PP which resulted in the equivalent clonogenic cell death, suggesting that UV-induced DNA damage was the primary cause of cell death (Figure 1).

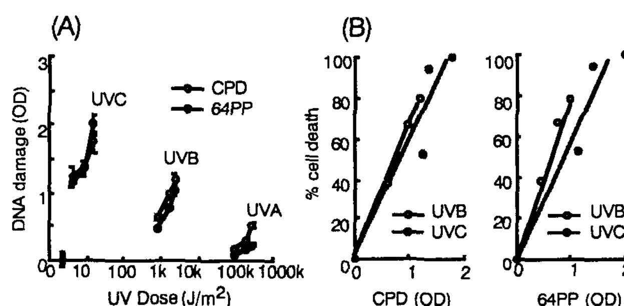

Figure 1. Formation of DNA photoproduct (A) and the linear correlation between the amount of DNA photoproduct and cell death (B) following the UVB or UVC irradiation.

When cells were irradiated with UVB at $3200J/m^2$ and UVC at $16J/m^2$, a rapid and transient phosphorylation of ERK and a sustained phosphorylation of p38 were observed. The phosphorylation of p53 at Ser15 and at Ser392 residues were also observed in 2 h after the irradiation, followed by the accumulation of p53 protein. The phosphorylation at Ser15 was diminished by wortmannin, a phosphoinositide 3-kinase inhibitor that blocks signals from DNA damage to

p53. In contrast, an antioxidant N-acetyl-cysteine and a p38 inhibitor SB203580 suppressed only Ser392 phosphorylation, suggesting that UV-induced oxidative stress and p38 activation were involved in the phosphorylation of this site (Figure 2).

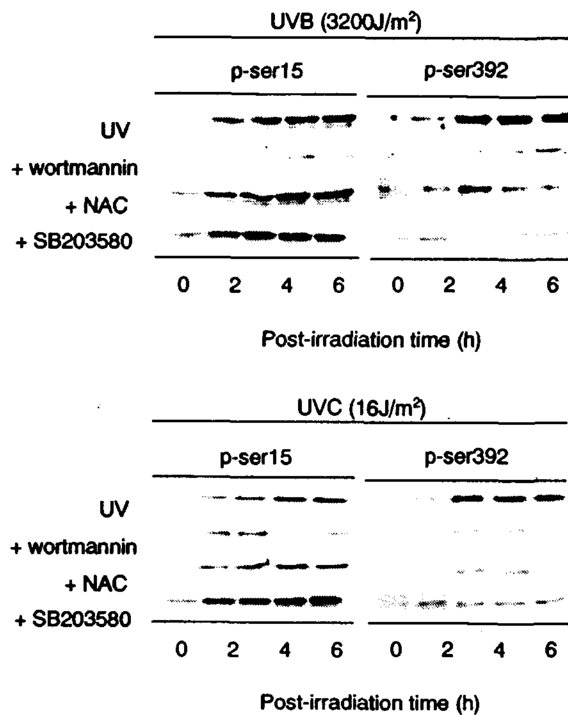

Figure 2. Inhibition of p53 phosphorylation at Ser15 and at Ser392 by wortmannin (PI 3-k inhibitor), N-acetyl-cysteine (antioxidant) and SB203580 (p38 inhibitor) in UVB or UVC irradiated cells.

Regarding the apoptic signals, the amount of cytochrome C in the cytosol fraction started to increase in 4 h after the UVB or UVC irradiation and maintained the high level up to 24 h. Approximately 22% of total cells exhibited Annexin V-positive in 6 h following the irradiation.

Overall, no difference was found in chronological responses of MAPK, p53, and apoptic signals between UVB-irradiated and UVC-irradiated cells. These results suggested that DNA damage-independent intracellular signaling molecules similarly responded to UVB and UVC when the equal level of DNA photoproducts were generated.

REFERENCES

1. Lakin N.D. and S P. Jackson (1999) Regulation of p53 in response to DNA damage. *Oncogene* 18, 7644-7655.

2. Shaulian E., M. Schreiber, F. Piu, M. Beeche, E.F. Wagner and M. Karin. (2000) The mammalian UV response: c-Jun induction is required for exit from p53-imposed growth arrest. *Cell* 103, 897-907.

3. Matsuda, N., M. Horiwaka, L-H. Wang, M. Yoshida, K. Okaichi, Y. Okumura and M. Watanabe (2000) Differential activation of ERK 1/2 and JNK in normal human fibroblast-like cells in response to UVC radiation under different oxygen tensions. *Photochem. Photobiol.* 72, 334-339.

4. Mori, T., M. Nakane, T. Hattori, T. Matsunaga, M. Ihara, O. Nikaido (1991) Simultaneous establishment of mono-clonal antibodies specific for either cyclobutane pyrimidine dimer or (6-4) photoproduct from the same mouse immunized with ultraviolet-irradiated DNA. *Photochem. Photobiol.* 54, 225-232.