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UV-responsive intracellular signaling pathways: MAPK, p53, and their crosstalk

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There are two distinct UV-responsive signaling pathways in UV-irradiated mammalian cells, i.e., the DNA damage-dependent and -independent pathways. The former occurs in nucleus and results in growth arrest and apoptosis via post-translational modification of p53. The latter is initiated by oxidative stress and/or by damages in cell membrane or cytoplasm, which activate signaling cascade through intracellular molecules including mitogen activated protein kinases (MAPK). In normal human fibroblastic cells, all of MAPK family members, extracellular signal-related kinases (ERK), c-Jun N-terminal kinases (JNK) and p38, were rapidly phosphorylated following UV-irradiation. ERK phosphorylation was suppressed by an inhibitor of receptor tyrosine kinases (RTK). As ERK usually responds to mitogenic stimuli from RTK ligands, UV-induced ERK phosphorylation may be linked to the proliferation of survived cells. In contrast, phosphorylation of JNK and p38, as well as apoptosis, were modulated by the level of UV-generated oxidative stress. Therefore, JNK and p38 may take part in oxidative stress-mediated apoptosis. Phosphorylation of p53 at Ser and Thr residues are essential for stabilization and activation of p53. Among several sites reported, we confirmed phosphorylation at Ser-15 and Ser-392 after UV-irradiation. Both of these were inhibited by a phosphoinositide 3-kinase inhibitor, presumably due to the shutdown of signals from DNA damage to p53. Phosphorylation at Ser-392 was also sensitive to an antioxidant and a p38 inhibitor, suggesting that Ser-392 of p53 is one of the possible points where DNA damage-dependent and -independent apoptic signals merge. Thus, MAPK pathway links UV-induced intracellular signals to the nuclear responses and modifies DNA damage-dependent cellular outcome, resulting in the determination of cell death.

Key words : UV, oxidative stress, MAPK, p53, apoptosis

INTRODUCTION

The exposure of mammalian cells to UV radiation results in DNA damage such as the formation of cyclobutane pyrimidine dimers and (6-4) photoproducts by direct excitation of bases, which can lead to growth arrest and cell death through activation of various signaling molecules including p53. The signals originated from DNA damage induce accumulation of p53 through post-translational modifications, i.e., phosphorylation or acetylation, followed by an elevated expression of p53-regulated genes encoding specific proteins for apoptosis and cell cycle arrest [1].

Besides the DNA damage, UV radiation generates reactive oxygen species and activates cell membrane

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molecules such as receptor tyrosine kinases, which then trigger various signaling cascades [2]. Therefore, UV is able to elicit cellular responses through several intracellular molecules that are apparently not involved in damaging the DNA. Among the UV-responsive molecules, MAP kinases (MAPK) are activated by specific upstream molecules (MAPKK) on dual phosphorylation of threonine (Thr) and tyrosine (Tyr). Substrates of MAPK such as transcription factors are then phosphorylated and activated, which results in the transcription of specific genes [3]. Therefore, MAPK are the central elements of the signaling cascade that links UV-induced intracellular signals to the nuclear responses and modify DNA damage-originated cellular responses, resulting in the determination of cell fate.

In order to better understand the signaling network in UV-irradiated cells, we examined responses of MAPK as DNA damage-independent pathway, p53 as DNA damage-dependent pathway, and the interaction of these molecules in UV-irradiated normal human fibroblastic cells.

MATERIALS AND METHODS

Normal human embryonic fibroblast-like cells (HE49) 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. For hypoxic culture, we used a multigas incubator in which cells were cultured in a humidified atmosphere containing 5% CO2-5% O2. The pO2 in the normoxic or hypoxic culture medium was 144.5mmHg and 41.5mmHg, respectively. UV irradiation was performed using the 254-nm source with a dose rate of $1J/m^2/sec$.

Phosphorylation and activation of MAPK family members, extracellular signal-related kinases (ERK), c-Jun N-terminal kinases (JNK) and p38, were examined by western blot analysis using antibodies against phosphorylated MAPK and kinase assay using [γ-^{32}P]-ATP, respectively [4]. The amount of p53 and its phosphorylated forms were also detected by western blot analysis using antibodies against p53, p53 phosphorylated at Ser15 and at Ser392.

The intracellular oxidative level was visualized using dihydrorhodamine 123 [5]. Clonogenic cell death was determined by colony formation. Apoptosis was examined by western blot analysis for determination of mitochondrial cytochrome C release, Annexin V staining for detecting phosphatidylserine on cell membrane, and agarose gel electrophoresis for visualizing DNA fragmentation. Cyclobutane pyrimidine dimers (CPD) and (6-4) photoproducts (64PP) were quantitated by ELISA [6].

RESULTS AND DISCUSSION

When normoxic cells were irradiated with $16J/m^2$ of UV, the intracellular oxidative level was substantially increased at 30 min after exposure. The hypoxic condition and the presence of an antioxidant (N-acetyl-cysteine; NAC) effectively reduced this UV-induced increase in the intracellular fluorescence intensity. Thus, oxidative stress was produced differently by UV irradiation in normoxic, hypoxic and NAC-treated cultures.

UV irradiation induced rapid and transient phosphorylation of ERK and a sustained phosphorylation of JNK and p38. The elevated level of ERK activity was diminished by the presence of suramin, a receptor tyrosine kinase (RTK) inhibitor. In contrast, JNK activation was abrogated in hypoxic cultures and in NAC-treated cultures (Figure 1). These results suggested two different possible links; between RTK and ERK, and between oxidative stress and JNK.

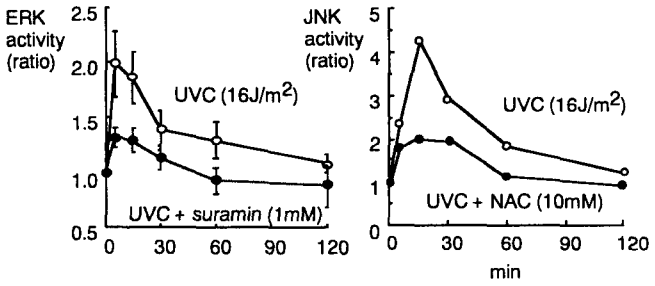

Figure 1. Activation of ERK and JNK following irradiation with UV.

At 24 h after irradiation, the DNA ladders in UV-irradiated hypoxic and NAC-treated cultures were apparently shorter than those of normoxic cultures. Furthermore, cell survival after irradiation with UV was increased in hypoxic and NAC-treated cultures compared to normoxic cultures. These findings raised the following possibilities; 1) The UV-generated oxidative stress is responsible for DNA damage-independent intracellular signaling through the JNK pathway rather than ERK pathway. 2) Different oxygen tensions in UV-irradiated cultures result in different balance between activities of ERK and JNK. 3) These changes of balance may contribute in some part to the level of apoptosis and cell survival after UV-irradiation.

Since it is evident that the lethal effect of UV radiation on cells is mainly attributable to DNA damage, the formation of DNA photoproduct and the response of p53 were then examined. 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.

Phosphorylation of p53 at Ser15 and at Ser392 residues were observed in 2 h after the irradiation, followed by the accumulation of p53 protein. This phosphorylation at Ser15 was diminished by wortmannin, a phosphoinositide 3-kinase inhibitor that blocks signals from DNA damage to p53. In contrast, phosphorylation at Ser-392 was suppressed not only by wortmannin but also by NAC and p38 inhibitor, SB203580. Therefore, signaling molecules in DNA damage-independent pathway, UV-generated oxidative stress and p38, were implicated to take a part in the phosphorylation of p53 at Ser392 (Figure 2).

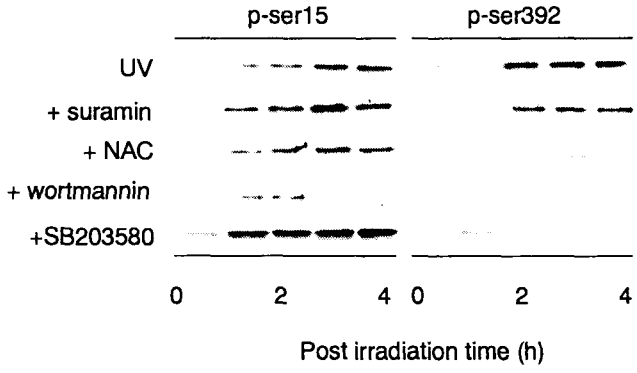

Figure 2. Phosphorylation of p53 at Ser15 and at Ser392 residues.

After the activation of MAPK and p53 pathways, the apoptic signals were then detected chronologically. The amount of cytochrome C in cytosol fraction started to increase in 4 h, approximately 22% of total cells exhibited

Annexin V-positive in 6 h, and DNA ladder formation appeared in 16 h following the irradiation.

The possible UV-responsive signaling network based on these findings, also including the results from other investigations, are illustrated in Figure 3. The DNA damage formed by UV are detected by sensory molecules and converted to the intracellular signal, which is conveyed by phosphoinositide 3-kinases, presumably ATR. Then phosphorylation and stabilization of p53 take place. In parallel to this DNA damage-dependent pathway, UV-generated oxygen stress triggers activation of JNK, and probably p38, pathways. The activated p38 may phosphorylate p38 at Ser392. Thus, this is one of the possible points where DNA damage-dependent and in-dependent apoptic signals merge. The activated p53 then regulates apoptosis or cell cycle arrest. While the clonogenic cell death may be a consequence of apoptosis and replicative death due to the prolonged cell cycle arrest, the cell survival results from repair of damaged cells, coupled with the mitogenic signal from RTK and ERK which generally respond to mitogenic stimuli. Thus, MAPK pathway links UV-induced intracellular signals to the nuclear responses and modifies DNA damage-dependent cellular outcome, resulting in the determination of cell death.

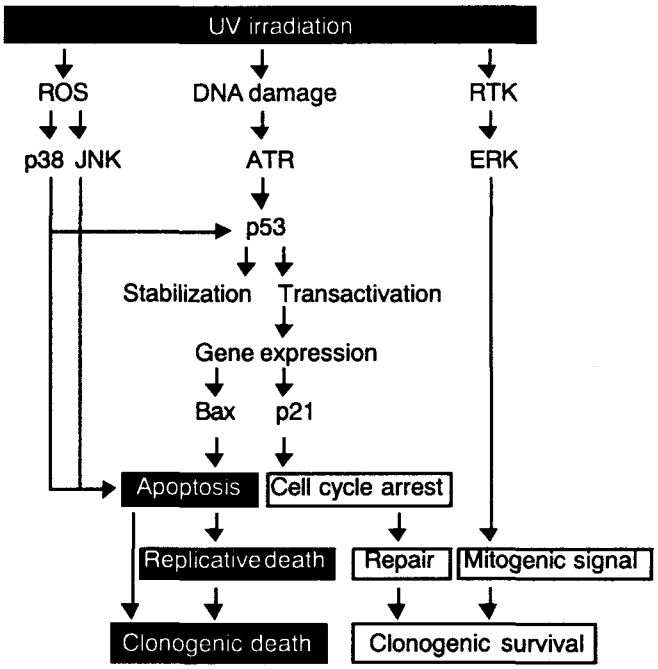

Figure 3. Overview of UV-responsive signaling cascade including DNA damage-independent MAPK and DNA damage-independent p53 pathways.

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. Seger, R. and E.G. Krebs (1995) The MAPK signaling cascade. *FASEB J.* 9, 726-735.

4. Matsuda, N., N. Morita, K. Matsuda and M. Watanabe (1998) Proliferation and differentiation of human osteoblastic cells associated with differential activation of MAP kinases in response to epidermal growth factor, hypoxia, and mechanical stress in vitro. *Biochem. Biophys. Res. Commun.* 249, 350-354.

5. Huang, R., J.-X. Wu, Y. Fan and E. Adamson (1996) UV activates growth factor receptors via reactive oxygen intermediates. *J. Cell Biol.* 133, 211-220.

6. Mori, T., M. Nakane, T. Hattori, T. Matsunaga, M. Ihara, O. Nikaido (1991) Simultaneous establishment of monoclonal 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.