Fas-independent Apoptosis Induced by UVC in p53-Mutated Human Epithelial Tumor A431 Cells through Activation of Caspase-8 and JNK/SAPK

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(Received on January 23, 2001)
(Revision received on May 7, 2001)
(Accepted on May 10, 2001)

A431 cells/UVC-induced apoptosis/Caspase 8/Fas/JNK/PAPK.

We previously observed that p53-mutated human epithelial tumor A431 cells underwent apoptosis after ultraviolet C (UVC) irradiation through the caspases-8 and -3 pathway. Fas/FasL is known to initiate apoptosis in several cell lines via caspase-8 activation. Then, to determine if Fas/FasL mediates apoptosis in A431, we investigated Fas expression and modulation in UVC-irradiated A431 cells. A431 constitutively expressed Fas, which gradually decreased after UVC-irradiation. Pretreatment with a neutralizing anti-Fas antibody, ZB4, did not abrogate the UVC-induced apoptosis. An agonistic anti-Fas antibody, CH11, very slowly induced apoptosis in A431, suggesting that the constitutively expressed Fas had a low functional potential. Hence, UVC-induced apoptosis in A431 seems to occur independent of the Fas signal. Interestingly, however, a pretreatment with CH11 remarkably potentiated UVC-induced apoptosis. An inhibitor of caspase-8, Ac-IETD-CHO, partially inhibited UVC-induced apoptosis. JNK was phosphorylated immediately after exposure to UVC, prior to apoptotic chromatin condensation. Our data suggest that the activation of caspase-8 occurs independent of Fas upregulation, and that JNK/SAPK contributes to UVC-induced apoptosis in human epithelial A431 cells.
INTRODUCTION

Apoptosis plays a critical role in a variety of pathological and physiological phenomena of skin cells\(^1,2\). Human skin is directly exposed to ultraviolet light (UV) in sunlight, and the UV often causes damage to skin cells. For a long time DNA has been regarded as the major molecular target for UVB (290–320 nm) and UVC (200–290 nm)\(^1,3\). Apoptosis in response to DNA injury is dependent on the induction of wild-type cellular p53 protein\(^4,5\). UVB and UVC are known to elicit p53-dependent apoptosis in DNA-damaged epidermal cells. However, p53-mutated epidermal cells, such as human epidermoid tumor cells, A431\(^6,7\) and human keratinocyte cell lines\(^8,9\), also undergo apoptosis following UV exposure.

Accumulated evidence shows that UV can also affect cytoplasmic and cell membrane structures and functions\(^2,3,10\), such as the activation/inhibition of receptors, ceramide formation and the activation of PKC. These processes seem to play predominant roles in mediating stress-induced apoptosis in p53-mutated cells. The A431 human epidermal cell line carries exclusively a mutant form of the p53 gene, and has been utilized as a material for studying UV- and radiation-induced apoptosis in p53-mutated cells\(^6,7,11,12\). In a preceding paper, we reported that caspase-8 and -3 cascade contributed to UVC-induced apoptosis in A431 cells\(^13\).

Caspase-8 is known as a key activator caspase in apoptosis mediated by Fas and other cell-death receptors\(^14–18\). Fas (CD95/APO-1) is a member of the tumor necrosis factor/nerve growth factor receptor family membrane receptor protein\(^15\). Upregulation of Fas or FasL expression plays a pivotal role in apoptosis induced by genotoxic agents, including UV, in a variety of cells, such as keratinocytes and lymphocytes\(^19–22\). Fas expression induced by radiation and UV has been observed to correlate directly to wild-type p53\(^4,23\). Furthermore, direct activation of Fas by its aggregation was shown to mediate UV-induced apoptosis in the p53-mutated human keratinocyte cell line HaCaT and SVHK cells upstream of the caspase-8 cascade\(^8,9\). The intracellular precise signaling mechanism responsible for UVC-induced apoptosis, however, remains ill-defined.

c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and/or p38 mitogen-activated protein kinase (MAPK) are known to be involved in apoptosis\(^24–27\). The JNK/SAPK pathway contributes to apoptosis by regulating FasL expression\(^27\). JNK kinase (MEKK1), a kinase upstream of JNK\(^28\), also plays a role in lymphocyte apoptosis by inducing FasL expression\(^29\). p38 MAPK, a subfamily of MAPK, regulates gene expression in response to various extracellular stimuli. In fact, SB203580, a highly specific inhibitor of p38 MAPK, suppresses UVB-induced apoptosis in human keratinocyte HaCaT\(^26\). However, JNK/SAPK, but not p38 MAPK, is persistently activated in radiation/UVC-induced apoptosis in Jurkat T cells\(^35\). Recently, Belka et al demonstrated that radiation induced a rapid activation of caspase-8, followed by apoptosis, independently of Fas/FasL interaction\(^30\). Using cells lacking Src-like tyrosine kinase (Lck), they concluded that radiation activated caspase-8 via a Lck-controlled pathway independently of FasL expression.

In the present study, we extended our previous study\(^13\) on the UVC-induced apoptosis in A431 cells to investigate the Fas/FasL system and protein kinase signaling. The results re-
vealed that UVC-induced apoptosis was independent of Fas, and that JNK/SAPK-phosphorylation occurred in the earliest phase of apoptosis.

**MATERIALS AND METHODS**

**Chemicals**

PD98059 (2'-Amino-3'-methoxyflavone), SB203580 (4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole), LY294002 (2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one), PP2 (4-Amino-5-(4-chlorophenyl)-7-(t-butyl)pyrozolo[3,4-d]pyrimidine), AG1478 (4-(3-Chloroanilino-6,7-dimethylquinoxaline) and Herbimycin were purchased from Calbiochem. (San Diego, CA, USA). Antibody kits of PhosphoPlus p38 MAP kinase and PhosphoPlus SAPK/JNK were obtained from New England Biolabs (Boston, MA, USA). Human anti-Fas antibodies CH11, UB2 and ZB4, were from MBL (Nagoya, Japan). All other reagents were of analytical grade.

**Cell culture**

The A431 cell line was obtained from the Health Science Research Resources (Osaka, Japan). Cells were cultured as previously described[13]. Briefly, cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 4 mM L-glutamine, 4.5 g/l glucose, 0.15% NaHCO₃, 50 units/ml penicillin, and 50 µg/ml streptomycin (Gibco-BRL, Rockville, MD, USA) in a humidified atmosphere containing 5% CO₂ at 37°C. The cells were seeded at 2 × 10⁵ cells per 35 mm culture dish with the growth medium and sub-cultured after growing to about 80% confluence using 0.25% trypsin (Gibco-BRL).

**UV-source and dosimetry**

The UV light source was a germicidal lamp (Toshiba GL10, Tokyo, Japan), which emitted UVC with the peak at 254 nm. The fluence rate was measured by a UV digital radiometer (Model UVX, UVP Inc. Upland, CA, USA) with a Model UVX-25 sensor[31].

**UVC irradiation and treatment with chemicals**

Cells were kept for at least 2 h after reseeding of 80% confluent cells to adhere to dishes. The medium was removed prior to irradiation, and the adhered cells were exposed to UV light at various doses, ranging from 0 to 50 J/m². Chemicals at various concentrations were added to the cells 2 h after reseeding. For irradiation of the chemical-treated cells, irradiation was performed 1 h after the treatment, as described above.

**Evaluation of apoptosis**

The number of apoptotic cells was scored[32] after fixation with 1% glutaraldehyde (TAAB Lab. Ltd., Berks, UK) in 0.1 M phosphate buffer (pH 7.4) and subsequent staining with 0.3 mM Hoechst 33342. Cells with the condensed chromatin were judged as apoptotic cells by fluorescence microscopy.
Flow cytometric detection of Fas expression

Fas expression was determined with flow cytometry essentially according to the method of Leverkus et al. After being detached from the plates by a brief treatment with 0.25% trypsin, cells (1 \times 10^6) were treated with a monoclonal Fas antibody, ZB4, followed by FITC-conjugated rabbit anti-mouse F(ab')2 fragments (Dako Japan, Kyoto, Japan). Cells were then flow cytometrically analyzed by an Epics XL model (Beckman Coulter: Miami, FL, USA) and data were processed by CELL Quest software (Becton-Deckinson Co., San Jose, CA. USA).

Western blot analysis

Western blotting was performed according to the protocol provided by the manufacturer. Briefly, A431 cells (1 \times 10^7) irradiated by UVC (20 J/m^2) were harvested by scraping at given times with the lysis buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% w/v SDS, 10% glycerol, 50 mM DTT. The lysates were boiled at 100°C for 10 min and centrifuged at 12,000 rpm for 30 min at 4°C. The supernatant protein (20 µg) was applied to electrophoresis on 12.5% SDS-polyacrylamide gel. After electro-transfer using the BIO-RAD system (Hercules, CA. USA), the membrane was probed with the primary antibodies and then anti-rabbit HRP-IgG. Detection was performed with the enhanced chemiluminescence (ECL) system (Amersham, Bucks. U.K) according to the manufacturer’s protocol.

RESULTS

Induction of apoptosis after UVC-exposure

A431 cells exhibited the typical apoptotic chromatin condensation with nuclear fragmentation after UVC exposure. Fig. 1 shows the time- and dose-dependent increase in apoptosis judged by chromatin condensation after UVC-irradiation. Apoptotic cells increased rapidly after irradiation until 12 h. Thereafter, the percentages of apoptotic cells rose gradually and reached to about 90% after 24 h when irradiated at 50 J/m^2. UVC doses higher than 5 J/m^2 induced apoptosis in a dose-dependent manner.

These results clearly indicate that A431 cells undergo apoptosis within 24 h following UVC-irradiation.

Fas expression after UVC-irradiation

Our previous results suggested the involvement of caspase-8 in the UVC-induced apoptosis of A431 cells. One of the most defined upstream mechanisms for caspase-8 activation is the Fas/FADD cascade. Therefore, we examined changes in Fas expression in UVC-irradiated A431 cells (Fig. 2). A Western blot analysis using UB2 anti-Fas antibody (Fig. 2A) revealed the constitutive expression of Fas in A431 cells; and the expression did not change appreciably until 4 h after 20 J/m^2 UVC-exposure. The constitutive expression of Fas as well as the absence of Fas upregulation was further confirmed by flow cytometry using the ZB4 anti-Fas antibody. As shown in Fig. 2B, Fas expression following UVC exposure did not increase, but rather gradually decreased until 12 h. These results strongly suggest that the
Fig. 1. Time- and dose-dependent induction of apoptosis by UVC-irradiation. Apoptotic cells were scored as described in the Materials and methods section after the incubation times given on the abscissa. The closed circles with a broken line, closed triangles with a solid line, open circles with a fine dotted line and closed squares with a dotted line represent the data for the cells irradiated by UVC doses 5, 10, 20 and 50 J/m² respectively.

UVC-induced apoptosis in A431, in which activation of caspase-8 was involved, is independent of Fas upregulation.

**Induction of apoptosis by an anti-Fas antibody, CH11 with or without UVC**

To further explore the function of the constitutively expressed Fas, we examined the effect of various concentrations (100 to 1000 ng/ml) of an agonistic anti-Fas antibody, CH11, which had an apoptosis-inducing ability (Fig. 3). The induction of apoptosis by CH11 alone was very slow, and rose gradually from 24 h to 72 h. The percentages of apoptotic cells remained at a low level until 24 h, and then increased in a dose-dependent manner. It therefore seems that the consitutively expressed Fas in A431 cells has a functionally low activity and mediates apoptosis very slowly after a treatment with CH11.

Interestingly, however, a pretreatment of the cells with CH11 remarkably stimulated the UVC-induced apoptosis (Fig. 4). In contrast to a few percent apoptosis induced by CH11 alone by 12 h, a significant increase in apoptosis was observed even at 4 h by exposure to UVC as low as 5 J/m². At a dose of 20 J/m², UVC induced 23% and 55% apoptosis after 4 h and 12 h, respectively. The pretreatment of cells with CH11 elevated these figures to 47% and
Fig. 2. Expression of Fas before and after UVC-irradiation (20 J/m²). Cont. in the figures represents data for the non-irradiated control cells. (A) Western blot analysis of cell lysates using human anti-Fas-antibody, UB2. The analysis was performed at the times shown at the upper part of the figure. (B) Flow cytometric analysis using anti-Fas-antibody, ZB4. Cell counts were plotted against the fluorescence intensities of the antibody-FITC. on Fl-1 channel of the Epics XL given on abscissa. Analyses were performed at the times given at the upper-left in each panel. The data shown as open area marked with Ab- on the upper 2 panels represent the results without the anti Fas antibody.
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Fig. 3. Time-dependent induction of apoptosis (chromatin condensation) by an anti-Fas antibody, CH11. Cells (2 × 10^5 cells/35 mm dish) were incubated with CH11 at various concentrations (closed circles with a solid line, 0 ng/ml; closed squares with a broken line, 100 ng/ml; closed diamonds with a broken line, 200 ng/ml; closed triangles with a dotted line, 500 ng/ml; reversed closed triangles with a dotted line, 1000 ng/ml). Apoptotic cells were counted after Hoechst 33342 staining following the incubation time given on the abscissa.

91%, respectively. Thus, CH11 can potentiate UVC-induced apoptosis under the condition used here.

UVC-induced apoptosis independent of Fas expression

To clarify the mechanism of the UVC-induced apoptosis and its remarkable enhancement by an anti-Fas antibody, CH11, we further examined the effect of neutralizing an anti-Fas antibody, ZB4, which had no ability to induce apoptosis (Fig. 5). While UVC (20 J/m²)-induced apoptosis at 12 h was markedly enhanced by CH11 (column #1 vs. #2), the apoptosis was not affected by a pretreatment with ZB4 (column #1 vs. #3). Furthermore, the enhancement of apoptosis by CH11 was abolished by the ZB4 treatment prior to a CH11 treatment (column #2 vs. #4). These results suggest that Fas does not contribute directly to UVC-induced apoptosis. Namely, UVC-induced apoptosis occurs independently of Fas-upregulation, at least until 12 h after UVC-exposure. However, potentiation of apoptosis by anti-Fas antibody indicates that Fas/FasL participates in the UVC-induced apoptosis of A431 cells under certain special circumstances, for instance under the influence of a CH11 treatment.
Furthermore, as shown by closed columns in Fig. 5, an inhibitor of caspase 8, Ac-Ile-Glu-Thr-Asp-CHO (Ac-IETD-CHO), markedly suppressed the UVC-induced apoptosis (20 J/m$^2$, 12 h) after a treatment of anti-Fas antibodies, either ZB4 alone or ZB4 + CH11, to about 30% compared to that observed without the inhibitor. Consequently, caspase-8 is involved in the apoptosis in A431 cells under the conditions used here. UVC plus CH11-enhanced apoptosis was inhibited to lesser extent, namely by about 50%. Hence, these findings further confirmed that UVC could induce apoptosis in A431 cells independently of the Fas/FasL system and the involvement of caspase-8.

Effects of signaling-protein kinase inhibitors

In order to characterize the signal-transduction pathway of UVC-induced apoptosis in A431 cells, the effects of inhibitors of MAPK and protein tyrosine kinase (PTK) were examined. Fig. 6 gives the results of preincubation for 1 h with various kinase-inhibitors on apoptosis following exposure to UVC (20 J/m$^2$).
**Fig. 5.** Effect of anti-Fas antibodies, CH11 and/or ZB4, and an inhibitor of caspase 8, Ac-IETD-CHO, on UVC-induced apoptosis. After a pretreatment with anti-Fas antibodies, CH11 (200 ng/ml) and/or ZB4 (500 ng/ml) for 2 h, cells were irradiated by UVC (20 J/m²). For a double treatment, 2-h of incubation with ZB4 was followed by CH11 addition. After a further 2-h incubation, cells were irradiated by UVC. To examine the effect of the caspase inhibitor, Ac-IETD CHO (100 ng/ml) was added 1 h before irradiation. The columns are numbered as shown at the bottom of the figure. The open/closed columns represent the data obtained without/with the caspase-8 inhibitor.

SB203580, a highly specific inhibitor of p38 MAPK, stimulated the apoptosis, suggesting that p38 MAPK suppresses apoptosis. On the other hand, LY294002, an inhibitor of PI3K and PD98059, an inhibitor of MEK1/2 in ERK pathway, suppressed the apoptosis. MEK1/2 in ERK pathway and PI3K, therefore, possibly participate, at least partially, in the signal transduction of the apoptosis.

To examine the possible involvement of PTK, we utilized three inhibitors with different specificity. Namely, AG1478, a selective inhibitor of epidermal growth factor receptor kinase, Herbimycin, an inhibitor of p60C-SRC and PDGF-induced phospholipase D, and PP2, a selective inhibitor of the Src family, including p56lck, were examined. As shown in the figure, these inhibitors had no significant effect on the UVC-induced apoptosis. Hence, PTK seems not to be involved in UVC-induced apoptosis in A431.

**Phosphorylation of JNK/SAPK after UVC exposure**

The activation (phosphorylation) of JNK/SAPK and/or p38 MAPK has been implicated in the pathway leading to stress-induced apoptosis in T-cells and keratinocytes. We thus, analyzed the expression of JNK/SAPK, phospho-JNK/SAPK and phospho-p38...
Fig. 6. Effects of various inhibitors of signaling protein kinases on UVC-induced apoptosis. Cells were irradiated by 20 J/m² after preincubation for 1 h with various chemicals in DMSO at concentrations of 10 µM SB203580, 20 µM LY294002, 20 µM PD98059, 100 µM AG1478, 800 µM Herbimycin and 50 µM PP2. Chemicals dissolved in DMSO had no effects on non-irradiated control cells.

MAPK after UVC-irradiation by Western blotting (Fig. 7). The expression of JNK/SAPK did not significantly change after UVC (20 J/m²) irradiation. Phosphorylated JNK/SAPK was not detected before exposure, but appeared immediately after irradiation, remained detectable and fluctuated with time. It therefore seems that JNK/SAPK activation (phosphorylation) is involved in UVC induced-apoptosis.

On the other hand, a phosphorylated form of p38 MAPK was constitutively detected in non-irradiated cells, and it gradually increased in a time-dependent manner after irradiation. This finding per se suggests the involvement of phosphorylated p38 MAPK in apoptosis. However, inhibition of this kinase by its specific inhibitor, SB203580, rather stimulated UVC-induced apoptosis, as shown in Fig. 6. The phosphorylation of the p38 MAPK, therefore, might not be directly related to apoptosis.
DISCUSSION

UV irradiation induces a rather complex cellular response, which eventually stimulates the apoptotic cell death program. In a previous study, we presented evidence to suggest the implication of a caspase-8 and -3 cascade in UVC-induced apoptosis in p53-mutated human epidermoid A431 cells. Here, we found that the Fas/FasL system was not involved in the apoptosis, and that the phosphorylation of JNK/SAPK played a role in the induction of apoptosis.

It has been shown that Fas upregulation following irradiation is dependent on wild-type p53 activity. After a treatment with DNA-damaging agents, the upregulation of Fas was observed only in cells with wild-type p53, not in cells with a mutant or without any p53. The absence of Fas upregulation in the p53-mutated A431 cells observed here is consistent with the above-mentioned reports.

The ineffectiveness of a neutralizing the Fas antibody, ZB4, in suppressing apoptosis (Fig. 5) confirmed that Fas/FasL was not involved in UVC-induced apoptosis. The neutralizing Fas antibody was also reported to fail to inhibit UVB-induced apoptosis in p53-transformed SVHK and p53-mutated HaCaT cells, although it effectively suppressed Fas-induced apoptosis in these cells. However, in SVHK and HaCaT cells, UVB directly stimulates Fas by its multimerization (or clustering) without its upregulation, and thereby activates the Fas pathway to induce apoptosis independently of the neutral ligand FasL.

We did not examine Fas-multimerization in the present study. We found, however, that constitutively expressed Fas in A431 (Fig. 2) is different in functional ability from those in SVHK and HaCaT cells. Fas in A431 responded very slowly to the agonistic anti-Fas-antibody CH11 (Fig. 3), which rapidly induced apoptosis in SVHK and HaCaT cells. Based on these findings, we suppose that the functional ability of Fas or the quantity of Fas in A431 is...
lower compared to that in SVHK and HaCaT cells, and that the Fas/FasL system plays no significant role in UVC-induced apoptosis in A431.

Interestingly, however, the pretreatment of CH11 enhanced UVC-induced apoptosis (Fig. 5). Apoptosis induced by CH11 alone remained at a low level for 12 h (Fig. 4). However, a treatment with CH11 for 2 h prior to UVC exposure remarkably stimulated UVC-induced apoptosis, and the apoptosis occurred faster with lower doses, as shown in Fig. 4. Thus, an anti-Fas antibody, CH11, can sensitize A431 to UVC by facilitating UVC-induced apoptosis. Data are not presently sufficient to further discuss this finding. The mechanisms for this phenomenon remain to be elucidated.

Compared to the marked inhibition of UVC-induced apoptosis by the caspase-8 inhibitor, the extent of the inhibition of the CH11-enhanced UVC-apoptosis was slightly reduced (Fig. 5). This finding suggests a possibility that the CH11-enhanced part of UVC-apoptosis might be, at least in part, independent of caspase-8.

Recently, Belka et al reported caspase–8 activation independent of the Fas system following exposure to ionizing radiation. The overexpression of dominant negative-caspase-8 interfered radiation-induced apoptosis. Further, caspase-8 activation and apoptosis by ionizing radiation were abrogated in cells genetically defective for the Src-like tyrosine kinase Lck. These findings indicate the importance of the Lck-controlled pathway for the radiation-induced activation of caspase-8 and apoptosis in Jurkat cells. An inhibitor of Src-like PTK, PP2, did not affect UVC-induced apoptosis in A431 cells (Fig. 6), indicating the independence of the apoptosis from the Lck pathway. Therefore, UVC-induced apoptosis in A431 is possibly different in the signal pathway from radiation-induced apoptosis in Jurkat cells.

We observed persistent activation of JNK/SAPK in A431 cells after UVC-irradiation (Fig. 7). JNK/SAPK, but not p38 MAPK, was also persistently activated in γ-ray- and UVC-induced apoptosis in human Jurkat T cells. Furthermore, the overexpression of JNK1 caused cell death in the transfected cells and the expression of dominant negative mutants of MAPK kinase 1 or JNK 1 prevented UVC-induced cell death.

Caspase 3, itself, has been reported to amplify the activation of the JNK/SAPK pathway, because it is able to cleave and activate MEKK1, a kinase upstream of JNK/SAPK. Using Jurkat cells transfected with the dominant active (DA)-MEKK1, Faris et al demonstrated that the expression of DA-MEKK1 resulted in an increased expression of FasL as well as in the apoptosis in parallel with prolonged JNK/SAPK activation. They also found that different types of stress, including UV, γ-ray, anisomycin and anti-FasAb, could induce apoptosis and prolonged JNK/SAPK activation. However, Fas-Fc protein could not inhibit UV and γ-ray induced apoptosis. From these results, they concluded that FasL was not critical for the induction of cell death by UV and γ-radiation.

Contrary to our result (Fig. 6), the pretreatment of HaCaT cells with SB203580, a highly specific inhibitor of p38 MAPK, suppressed UVB-induced apoptosis together with caspase-3 activation and the cleavage of poly(ADP) ribose polymerase. Therefore, UVC-induced apoptosis in A431 is distinct from UVB-induced apoptosis in HaCaT cells, in which p38 MAPK activation upstream of caspases plays an important role.

In A431 cells, epidermal growth factor receptors (EGFRs), tyrosine phosphorylation, is
increased in response to radiation, and an EGF treatment protects against radiation damage\textsuperscript{11}). In the present study, AG1478, a potent EGFR kinase inhibitor\textsuperscript{35}, did not inhibit UVC-induced apoptosis in A431 (Fig.6). Furthermore, EGF did not significantly affect apoptosis in A431 (data not shown). These results imply the independence of apoptosis from EGFR, unlike radiation-induced apoptosis in cells. The inhibitors of PTK, Herbimycin\textsuperscript{36} and PP2 discussed above also did not inhibit UVC-induced apoptosis, demonstrating that signaling via PTK might not participate in the signal transduction pathway in the UVC-induced apoptosis of A431. LY29400, an inhibitor of PI3K reduced resistance to UV-induced apoptosis in melanoma cells\textsuperscript{33}. PD98089, a selective inhibitor for MAPK/ERK1 that directly activates ERKs, inhibited UVB-induced apoptosis in JB6 cell\textsuperscript{34}. Both inhibitors partially suppressed the UVC-induced apoptosis in A431 cells (Fig. 6). Therefore, PI3K and MEK1/2 in the ERK pathway may contribute only partially to the signal transduction of apoptosis.

Based on the present findings, we propose the following scenario for UVC-induced apoptosis in A431 cells. UVC induces an immediate phosphorylation of JNK/SAPK, which initiates the apoptosis program via activation of caspases-8 and -3 independently of Fas upregulation.

**ACKNOWLEDGEMENT**

This work was supported in part by a Special Grant for Promoted Research from National Institute of Radiological Sciences.

**REFERENCES**

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