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Deregulation of Cyclin-Dependent Kinase 2 Activity Correlates With UVC-Induced Apoptosis in Chinese Hamster Ovary Cells

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Abstract Progression through the cell cycle relies on the activities of cyclin-dependent kinases (Cdk), which in turn are modulated by inhibitory proteins such as $p21^{(waf1/cip1)}$ that are induced when genomic damage occurs. In this study, we show that exposure of normal mammalian cells, such as NIH3T3 fibroblasts, to UVC (25 J/m^2, at 254 nm) induces the expression of p21 without causing significant apoptosis, whereas similar treatment of Chinese hamster ovary (CHO-K1) cells with UVC causes apoptosis without inducing p21. The absence of p21 in UV-irradiated CHO-K1 cells is accompanied by the deregulation of Cdk2 activity. The elevation of Cdk2 activity correlates with the increase of UV-induced apoptosis, which can be suppressed by small-molecule Cdk2 inhibitors such as roscovitine and pyrrolidine dithiocarbamate. The results of this study suggest that the deregulation of Cdk2 activity may be critical to UV-induced apoptosis in CHO-K1 cells. J. Cell. Biochem. 97: 824–835, 2006.

Key words: UVC; apoptosis; $p21^{(waf1/cip1)}$; cyclin-dependent kinase 2

In mammalian cells, exposure to ultraviolet (UV) radiation leads to the damage of cellular constituents, resulting in complex cell responses that include the induction of genes and the perturbation of various signaling pathways [Bender et al., 1997; Schwarz, 1998]. If damaged constituents in UV-irradiated cells cannot be repaired, cells are likely to die via the process of apoptosis. Apoptosis, which is widely observed in different types of cells in various organisms, has a unique morphological cell death pattern characterized by chromatin condensation, membrane blebbing, and DNA fragmentation [Kerr et al., 1972; Ellis et al., 1991].

We have previously reported that UVC irradiation (at 25 J/m^2) causes significant apoptosis in Chinese hamster ovary cells (CHO-K1) [Tzang et al., 1999b] and expression of the $p21^{(Waf1/Cip1)}$ gene is absent despite the induction of p53 and gadd45 in the UV-irradiated cells [Tzang et al., 1999a]. $p21^{(Waf1/Cip1)}$ is an universal inhibitor of cyclin-dependent kinases (Cdks), which is induced during DNA damage [Harper et al., 1993; Xiong et al., 1993; Dulic et al., 1994; Michieli et al., 1994]. $p21^{(Waf1/Cip1)}$ acts by binding to cyclin–Cdk complexes, and inhibiting their activity; it has been shown to be involved in cell-cycle arrest at the G_1 phase, presumably to allow time for the repair of damaged DNA [Gu et al., 1993; Harper et al., 1993; Xiong et al., 1993; Nakanishi et al., 1995]. Ectopic overexpression of human $p21^{(Waf1/Cip1)}$ markedly increased the survival rates of UV-irradiated CHO-K1 cells [Tzang et al., 2002]. However, Cdk activities in UV-irradiated CHO-K1 cells remain uninvestigated and the identity of the Cdk involved in UV-induced apoptosis of CHO-K1 cells is still unclear.

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In the present study, we demonstrate that Cdk2 but not Cdk4 activity is elevated in UV-irradiated CHO-K1 cells. The elevated Cdk2 activity correlates well with the increase of UV-induced apoptosis during the time course after UV irradiation. Inhibition of Cdk2 activity with chemicals such as roscovitine or pyrrolidine dithiocarbamate also suppresses the UV-induced apoptosis. A plausible signal pathway from the elevation of Cdk2 to the programmed cell death is hypothesized.

MATERIALS AND METHODS

Chemicals

Pyrrolidene dithiocarbamate (PDTC), glutathione (GSH), and DL-buthionine-[S,R]-sulfoximine (BSO) were obtained from Sigma (St. Louis, MO), and roscovitine was from Calbiochem (San Diego, CA).

Cell Cultures

CHO-K1 and NIH3T3 cells were originally obtained from the American Type Culture Collection (Manassas, VA). The CHO-K1 and NIH3T3 cells were maintained in 1× McCoy's 5A medium (Sigma) and 1× DMEM medium (Gibco, Grand Island, NY), respectively, supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and 0.03% glutamine, and were cultured at 37°C in a water-saturated atmosphere containing 5% CO_2.

UV Irradiation

Cells were washed with 1× PBS, and were exposed in uncovered dishes to UV using a germicidal lamp (254 nm, Sankyo Denki Co., Tokyo, Japan) in a UV box at dose of 25 J/m^2. The dose of UV irradiation was calibrated with a UV radiometer (UVP, Inc., San Gabriel, CA).

Western Blot Analysis

Western blot analysis was performed according to a standard protocol. Briefly, proteins were separated by SDS–polyacrylamide gel electrophoresis, transferred to PVDF membranes, and then probed with antibodies, such as $p21^{(Waf1/Cip1)}$ (sc-6264; Santa Cruz Biotechnology, Santa Cruz), Cdk2 (sc-6248; Santa Cruz), cyclin A (sc-596; Santa Cruz), cyclin D1 (sc-8396; Santa Cruz), Cdk4 (sc-260; Santa Cruz), actin (sc-1616; Santa Cruz), and phosphorylated Rb (R6275; Sigma). To detect the Ab–Ag complexes, an ECL detection kit (Amersham, Arlington Heights, IL) was used according to the instructions of the manufacturer.

Flow Cytometric Analyses of Apoptosis

Apoptosis was assayed using flow cytometric analysis either of the sub-G_1 population or of cells stained with Annexin V. (i) Sub-G_1: flow cytometric analysis of sub-G_1 cells was done according to a standard protocol. In brief, cells ($\sim 2 \times 10^6$) were fixed in 70% alcohol for 12–16 h at −20°C. The fixed cells were then incubated with RNase (10 μg/ml) and propidium iodide (PI, 50 μg/ml) at room temperature for 30 min prior to cell-cycle analysis with a flow cytometer (FACScan, Becton Dickinson, Franklin Lakes, NJ). (ii) Annexin V staining: Procedures for double staining of PI and Annexin V were used as described previously [Vermes et al., 1995]. Cells (about 1×10^6), after treatment and two washes with PBS, were incubated in buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) containing FITC-Annexin V and PI (both at 1 μg/ml) for 5 min at room temperature in the dark before analysis.

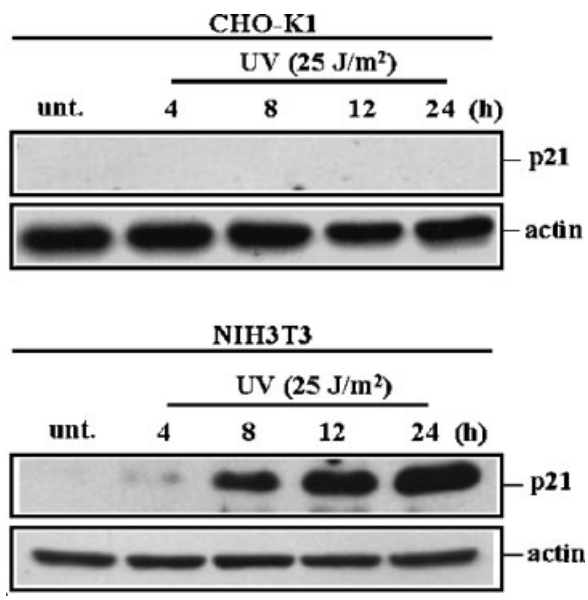
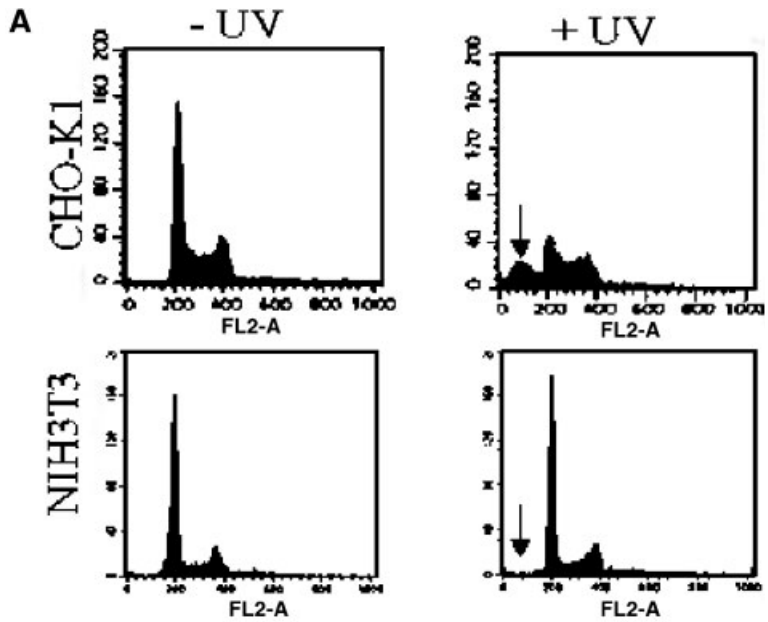


Fig. 1. Induction of $p21^{(Waf1/Cip1)}$ is not detected in CHO-K1 cells after UV treatment. Levels of $p21^{(Waf1/Cip1)}$ were examined by Western blot analysis in CHO-K1 and NIH3T3 cells at various time intervals (4–24 h) after UV treatment. Actin is used as a loading control. (unt = untreated cells).



Quantitative analysis of sub-G1 percentage		
	-UV	+UV
CHO-K1 cells	1.8 ± 0.3	22.4 ± 1.5
NIH3T3 cells	1.6 ± 0.3	1.9 ± 0.4

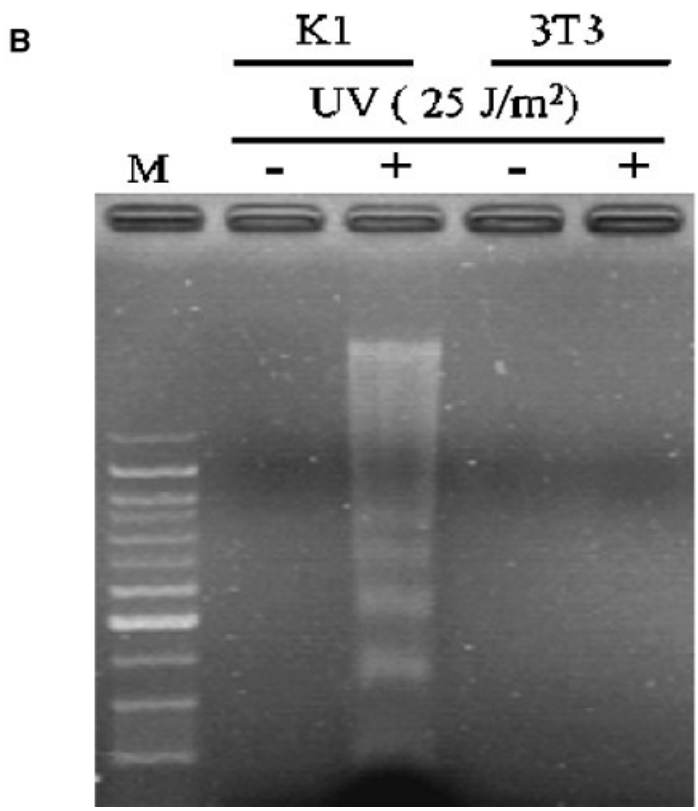


Fig. 2.

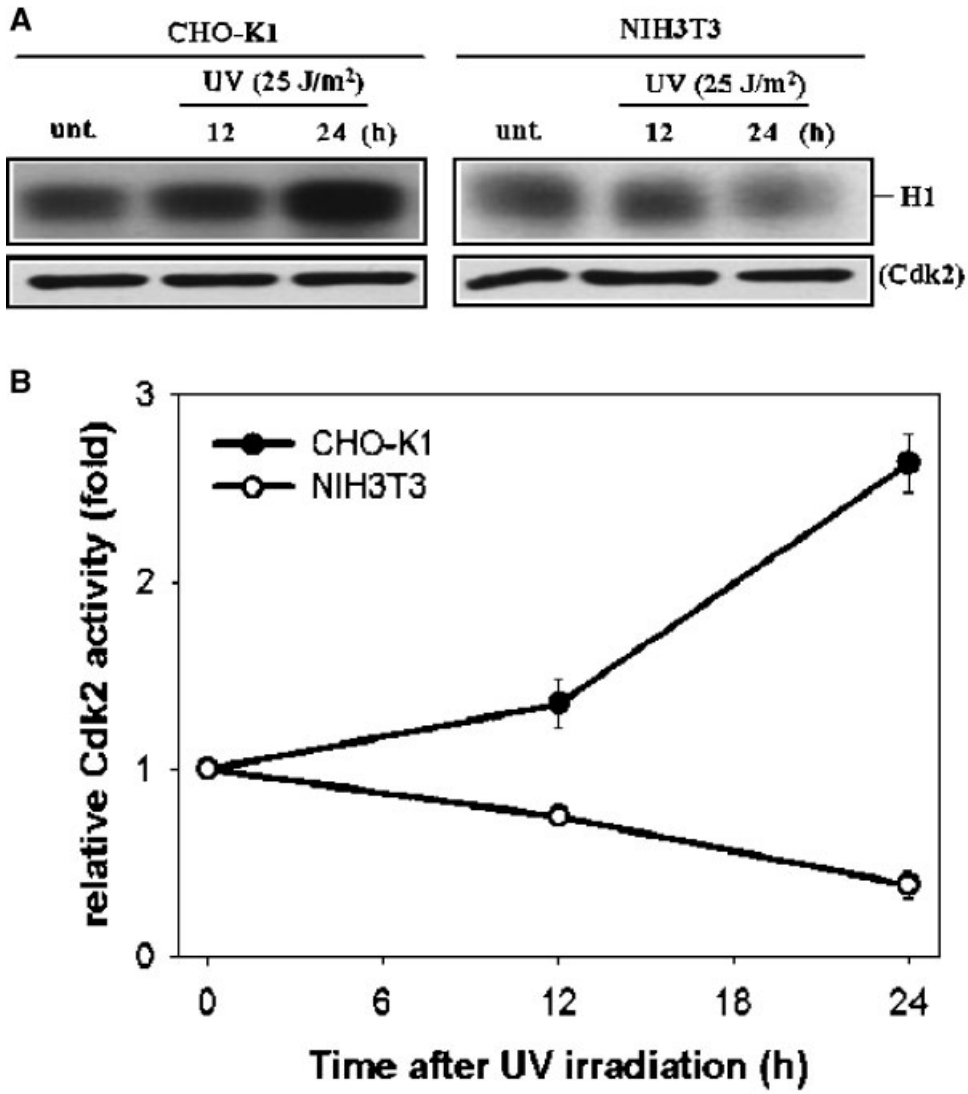


Fig. 3. Elevation of Cdk2 activity in UV-irradiated CHO-K1 cells. **A**: Kinase activity assays (H1, **upper panel**) of Cdk2 in CHO-K1 and in NIH3T3 cells at the indicated time intervals after UV irradiation. Amounts of Cdk2 immunoprecipitated during the assay were monitored by Western blotting with an anti-Cdk2 antibody as a loading control (**bottom**). **B**: Summary of quantitative analysis of Cdk2 activity. Data are expressed as means ± SE of at least three independent experiments.

DNA Fragmentation Assay

The DNA fragmentation assay was performed as described previously [Kondo et al., 1995]. In brief, cells after treatment were incubated in lysis buffer (10 mM Tris-HCl, 10 mM EDTA, and 0.2% Triton X-100, pH 7.5) for 10 min on ice. DNA in the supernatant after centrifugation (12,000*g* at 4°C for 30 min) of the lysis mixture was purified using standard procedures. The DNA samples were separated by electrophoresis on 1.5% agarose gels for the fragmentation assay.

Cdk Kinase Assay

Cdk2 or Cdk4 proteins were immunoprecipitated using the anti-Cdk2 or anti-Cdk4 antibodies and Protein A Sepharose (Amersham).

Fig. 2. UV induces apoptosis in CHO-K1 but not in NIH3T3 cells. At 24 h after UV irradiation, cells were harvested for measurement of apoptosis by (**A**) flow cytometric analysis, (**B**) DNA fragmentation analysis. The sub-G_1 fraction is marked with arrows. Quantitative analysis of sub-G_1 at 24 h after UV irradiation is summarized in the appended table. Data are shown as means ± SE of at least three independent experiments.

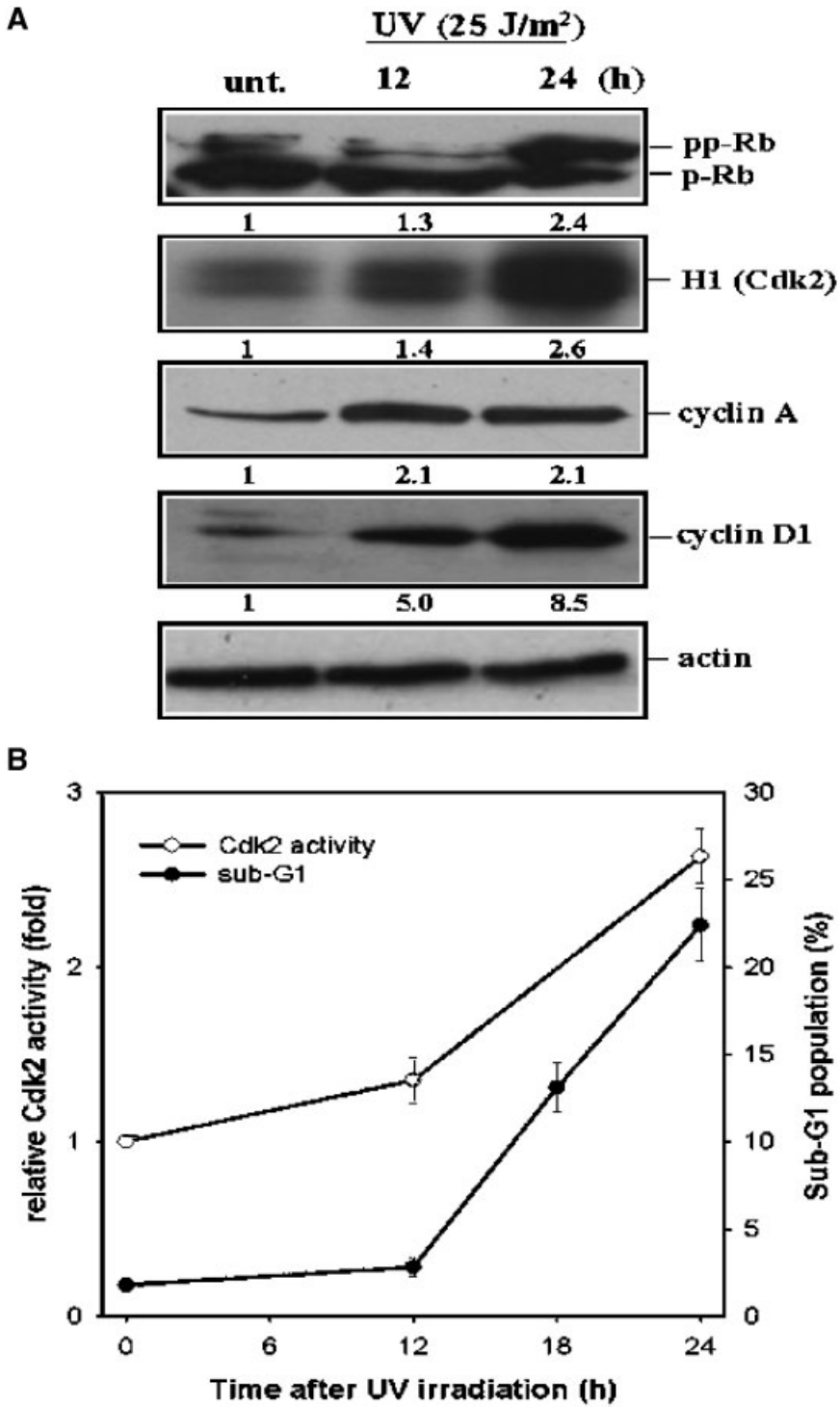


Fig. 4. Increase of phosphorylated-Rb in UV-irradiated CHO-K1 cells. **A**: Western blot analysis of Rb (p-Rb, hypophosphorylated-Rb; pp-Rb, hyperphosphorylated-Rb) and other cell cycle-related proteins (cyclin A, D1, and Cdk2). Cdk2 activity (H1) is shown as a control. **B**: Correlation of Cdk2 activity (open circles) and apoptosis (sub-G_1, close circles) with time following UV treatment (25 J/m^2).

Cdk2 or Cdk4 activities in the precipitated fractions were measured using histone H1 as an in vitro substrate in the presence of γ-^{32}P ATP, as described previously [Hiromura et al., 1999]. Contents of precipitated Cdk2 or Cdk4 were monitored by Western blotting using the anti-Cdk2 or Cdk4 antibodies as loading controls.

Measurement of Intracellular Reactive Oxygen Specie (ROS)

Intracellular oxidant levels were determined using 2,7-dichlorodihydrofluorescein (H_2DCF), which is oxidized in cells to the fluorescent dichlorofluorescein [Yang et al., 2001]. Cells were incubated with H_2DCF-diacetate (Molecular Probes, Eugene, Oregon) at 25 μM for 30 min at 37°C and were then washed with ice cold PBS. Measurements were performed by flow cytometric analysis with excitation at 488 nm and emission at 526 nm.

RESULTS

Deregulation of Cdk2 Activity Is Associated With UV-Induced Apoptosis in CHO-K1 Cells

Previously, we have shown that p21$^{(waf1/cip1)}$ mRNA was not detected in UV-irradiated CHO-K1 cells [Tzang et al., 1999a]. The absence of p21 expression was confirmed at the protein level by the Western analysis (Fig. 1), which shows that CHO-K1 cells, in contrast to the control NIH3T3 cells, have no p21 induction over the 24 h time course following UV irradiation. Moreover, CHO-K1 cells, but not NIH3T3 cells, underwent UV-induced apoptosis 24 h after the UV irradiation, as measured by the sub-G_1 fraction (Fig. 2A) or by the DNA ladder (Fig. 2B).

Since p21 is an inhibitor of Cdks, it is of interest to see the effect upon Cdk activity when p21 is absent in UV-irradiated cells. Indeed, the activity of Cdk2 was markedly elevated in UV-irradiated CHO-K1 cells 24 h following UV

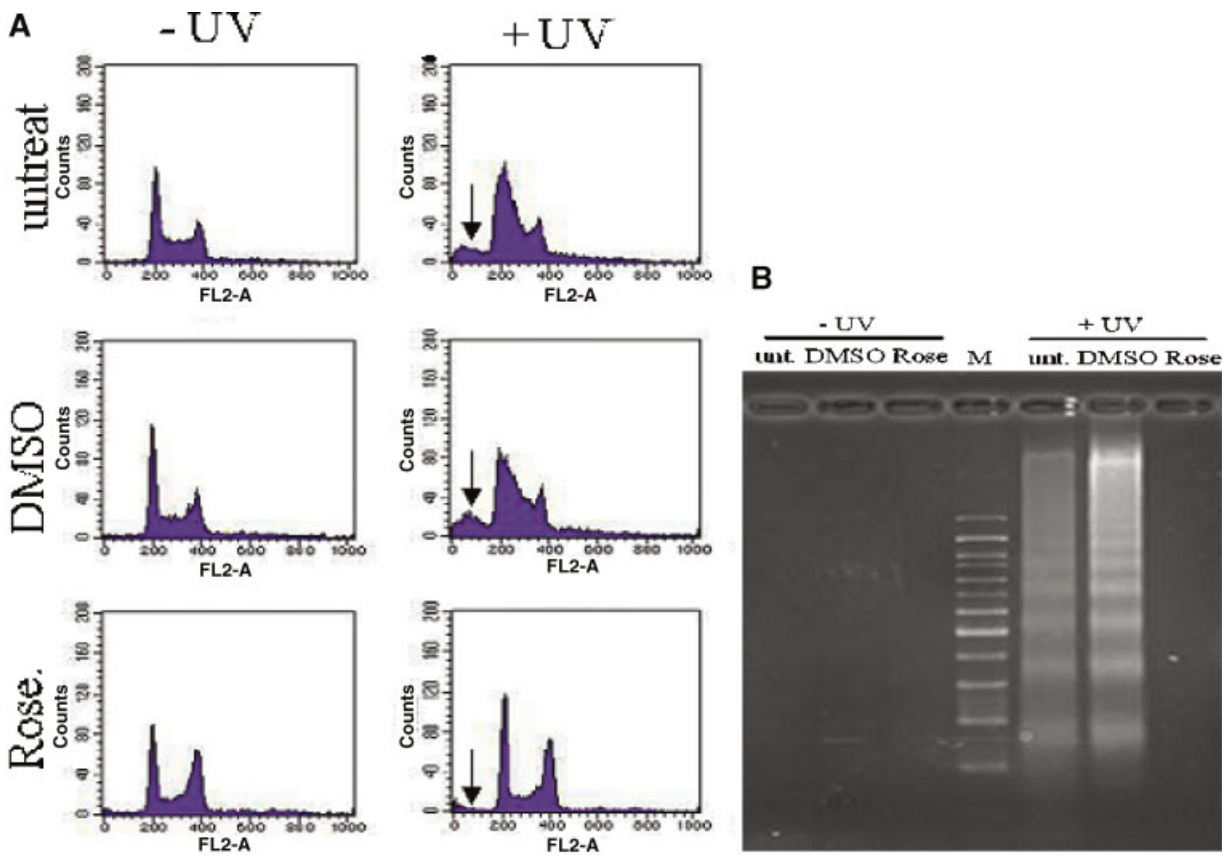


Fig. 5. Roscovitine inhibits UV-induced apoptosis in CHO-K1 cells. Cells were treated after UV irradiation with or without 180 μM roscovitine for 24 h and were harvested for measurement of apoptosis by (**A**) flow cytometric analysis, (**B**) DNA fragmentation analysis. The sub-G_1 fraction is marked with an arrow. (Rose = roscovitine). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

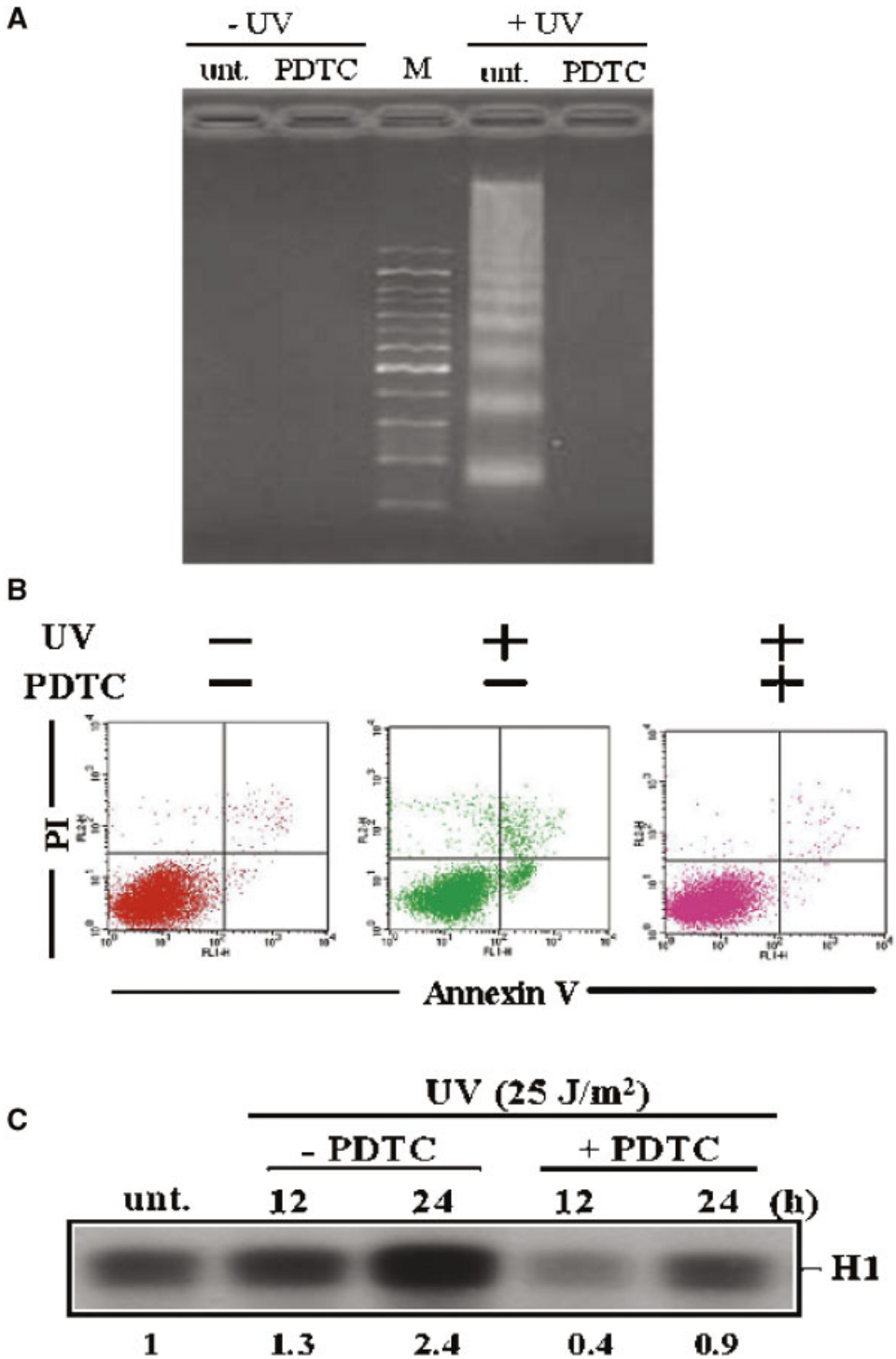


Fig. 6. PDTC inhibits UV-induced apoptosis in CHO-K1 cells. Cells were treated after UV irradiation with or without 5 mM PDTC for 24 h and were harvested for measurement of apoptosis by (**A**) DNA fragmentation analysis, (**B**) apoptotic analysis using a flow cytometer with PI and Annexin V staining. **C**: Kinase activity assay of Cdk2 at 12 and 24 h after UV irradiation with or without 5 mM PDTC. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

irradiation (Fig. 3A). Such an elevation of Cdk2 activity was not detected in NIH3T3 cells, where the activity of Cdk2 declined steadily after UV treatment (Fig. 3B), in agreement with the time course of p21 induction in those cells (Fig. 1). Consistent with the notion that Cdk2 phosphorylates the tumor suppressor Rb protein, the elevation of Cdk2 activity in UV-irradiated CHO-K1 cells was accompanied by an increase in Rb protein in its hyperphosphorylated state (Fig. 4A). The correlation between Cdk2 activity and UV-induced apoptosis is clearly indicated in the plot shown in Figure 4B.

Inhibition of Cdk Activity Is Accompanied by the Suppression of UV-Induced Apoptosis

To determine if the deregulation of Cdk activity is essential to the UV-induced apoptosis in CHO-K1 cells, we used roscovitine (a small molecule Cdk inhibitor) to test if inhibition of Cdk activities suppresses UV-induced apoptosis in CHO-K1 cells. Indeed, treatment with roscovitine clearly suppressed the UV-induced sub-G_1 fraction and the DNA fragmentation of CHO-K1 cells at 24 h following UV irradiation (Fig. 5A,B). Such suppression was not detected in cells treated with DMSO, the solvent control.

Interestingly, the antioxidant PDTC also effectively suppressed the UV-induced apoptosis of CHO-K1 cells, measured by the DNA ladder (Fig. 6A) and by cells stained for Annexin V (Fig. 6B, compare the lower corners at the right, with or without the drug). Moreover, the UV-induced elevation of Cdk2 activity was clearly inhibited by PDTC (Fig. 6C). To determine if the suppression of UV-induced apoptosis by PDTC was due to the effect of anti-oxidation, we performed similar experiments with GSH. GSH failed to suppress the UV-induced apoptosis (Fig. 7B, 26.6% of sub-G_1), although GSH reduced the level of reactive oxygen species (ROS) similar to PDTC (Fig. 7A, see the arrows leftward for the reduction of ROS). BSO, an inhibitor of endogenous GSH synthesis, increased ROS (Fig. 7A, arrow rightward for the increase of ROS) and did not enhance the UV-induced apoptosis (Fig. 7B, 22.9% of sub-G_1). Thus, the suppression of UV-induced apoptosis by PDTC is not likely due to its anti-oxidant effect. Taken together, the inhibition of Cdk activity is accompanied by the suppression of UV-induced apoptosis.

Cdk4 Activity Is Not Induced in UV-Irradiated CHO-K1 Cells

We showed above that Rb protein was found in its hyperphosphorylated state in UV-irradiated CHO-K1 cells. Since Rb protein can be phosphorylated by Cdk2 and by Cdk4/6, it is of interest to determine if Cdk4/6 activities are involved in phosphorylating Rb. Figure 8 shows that the activity of Cdk4 did not change during the time course following UV irradiation. Similar results were found for Cdk6 (data not shown). Thus, the increase of hyperphosphorylated Rb in UV-irradiated CHO-K1 cells is more likely due to the increase in Cdk2 activity and Cdk4/6 is probably not involved in the UV-induced apoptosis of CHO-K1 cells. This is consistent with the observation that ectopic expression of human $p16^{INK4a}$ did not suppress UV-induced apoptosis in CHO-K1 cells (data not shown).

DISCUSSION

When CHO-K1 cells are irradiated with UVC at 25 J/m^2, about 20% of the cells are apoptotic 24 h later, whereas when NIH3T3 cells are treated in the same manner, less than 2% of them are apoptotic at 24 h. The Cdk inhibitor, p21, is induced in UV-irradiated NIH 3T3 cells, but cannot be detected in CHO-K1 cells. This study addressed the relationship between activities, specifically Cdk2, and UV-induced apoptosis in CHO-K1 cells, and we show that Cdk2 activity and UV-induced apoptosis are closely correlated. We demonstrate that Cdk2 activity is elevated following UV-irradiation of CHO-K1 cells, and that suppression of Cdk2 activity with roscovitine or PDTC results in the reduction of UV-induced apoptosis. These data are consistent with our previous observation that ectopic expression of human p21 inhibits UV-induced apoptosis [Tzang et al., 2002]. We therefore hypothesize that deregulation of Cdk2 may lead to apoptosis. Deregulation of Cdk2 may initially result in excessive activation of the transcription factor E2F via the phosphorylation of Rb. Our data show that levels of hyperphosphorylated Rb are greatly increased in UV-irradiated CHO-K1 cells. Increase in hyperphosphorylated Rb would favor the release of E2F from the inhibitory binding of Rb and thus increase E2F activity. Excessive activation of E2F may cause apoptosis by p53-dependent or p53-independent routes [DeGregori et al., 1997; Dong

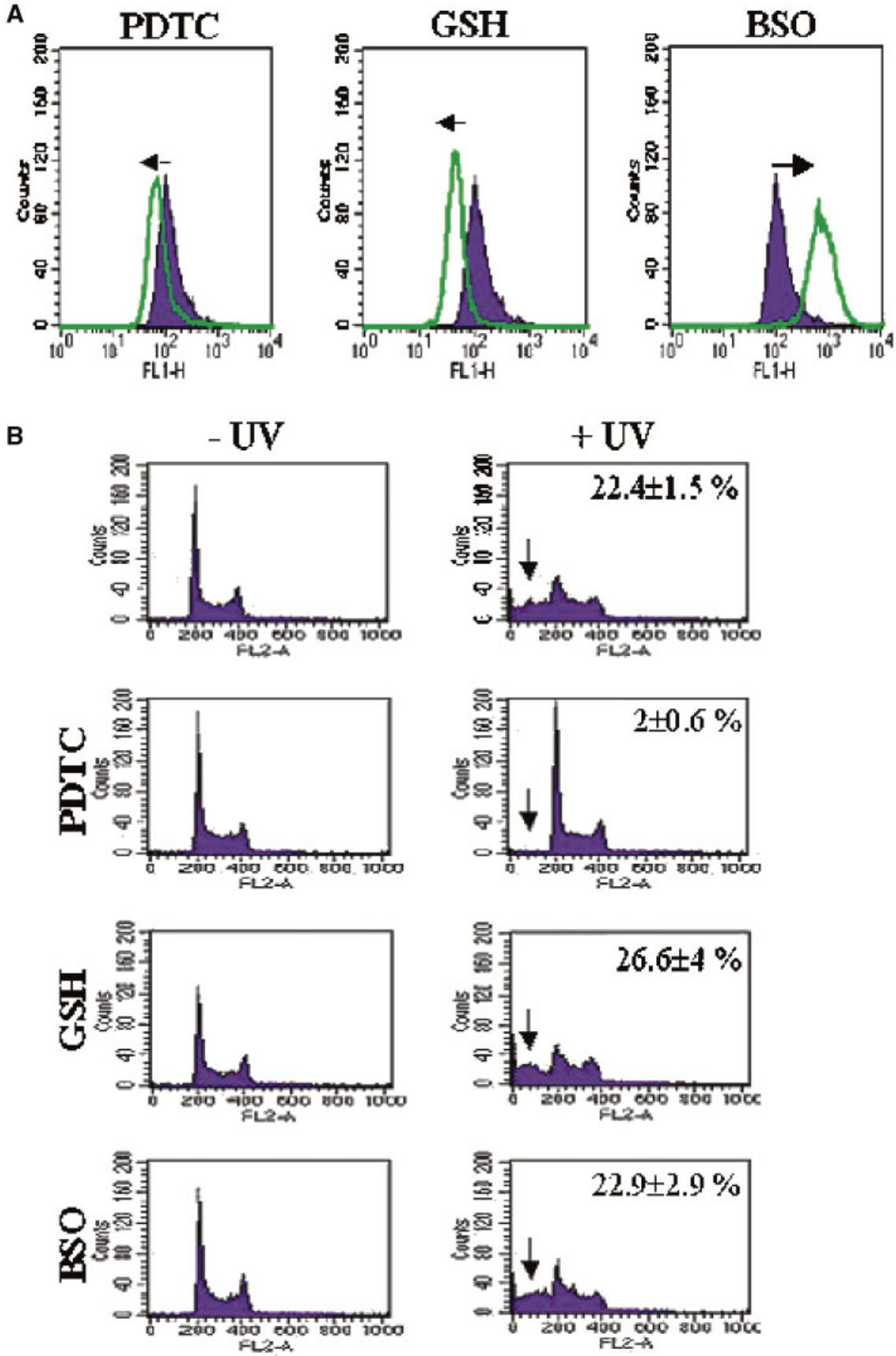

A
PDTC
GSH
BSO
Counts
FL1-H
B
- UV
+ UV
22.4±1.5 %
2±0.6 %
26.6±4 %
22.9±2.9 %
PDTC
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Fig. 7.

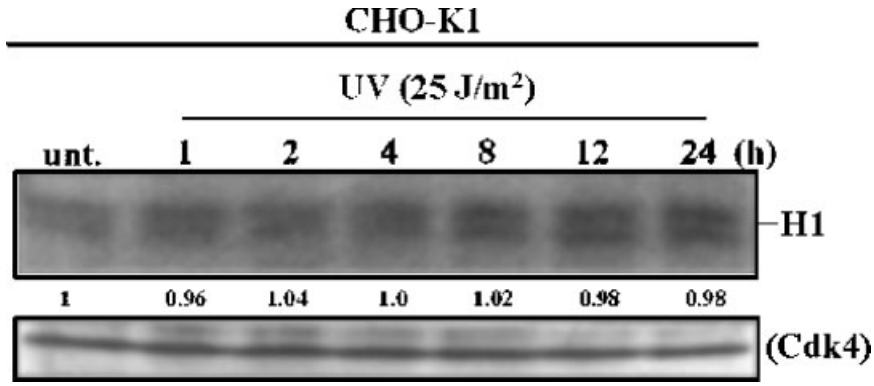

Fig. 8. No elevation of Cdk4 activity is seen in UV-irradiated CHO-K1 cells. Cdk4 activity in CHO-K1 cells at the indicated time intervals after UV irradiation was assayed (**upper panel**). Levels of Cdk4 immunoprecipitated during the kinase assay were monitored by Western blotting with an anti-Cdk4 antibody as a loading control (**bottom**).

et al., 1999, 2002; Elliott et al., 2001]. One of those routes involves the induction of $p14^{ARF}$, which in turn inhibits Mdm2 and as a result, the stability of p53 is enhanced and p53 accumulates.

Although the accumulation of p53 was detected in UV-irradiated CHO-K1 cells (data not shown), whether p53 plays a role in the UV-induced apoptosis of CHO-K1 cells is unclear. Nevertheless, a preliminary study using CHO-K1 cells transfected with a plasmid expressing mutant p53 ($273^{Arg \rightarrow His}$) to compromise the functions of wild-type p53 exhibited a similar extent of UV-induced apoptosis as did the parental cells (data not shown).

As mentioned earlier, overexpression of E2F may also lead to apoptosis via p53-independent routes. Induction of p73 by E2F has been related to certain types of DNA-damage induced apoptosis. A recent study showed that p73 might also be induced by UV [Lin et al., 2004]. We are currently testing the possible role of p73 in the UV-induced apoptosis of CHO-K1 cells.

It was noted that roscovitine caused accumulation of G_2/M (Fig. 5A). The effect of roscovitine has also been observed by others [Meijer et al., 1997]. It has been known that roscovitine inhibits activities of Cdk2 as well as Cdc2 (or Cdk1) with almost equal efficiency. Inhibition of Cdc2 activity results in G_2/M accumulation. Therefore, roscovitine in our study, very likely, inhibited both Cdk2 and Cdc2 activities. Thus, the reduction of UV-induced apoptosis by roscovitine might be attributed to the inhibition of Cdk2 and/or G_2/M arrest. However, our previous studies have shown that G_2/M arrest is ineffective to avoid UV-induced apoptosis [Tzang et al., 2002]. Cells pre-treated or immediately post-treated with colcemid are not more resistant to UV-induced apoptosis; in fact the apoptosis is exaggerated [Tzang et al., 2002]. Studies by others also show that G_2/M arrest does not protect cells from apoptosis [e.g., Hayashi et al., 2004]. Hence, the reduced apoptosis by roscovitine (Fig. 5) was more likely related to the inhibition of Cdk2 activity, but not the result of G_2/M arrest.

Although the elevation of Cdk2 activity has been shown to be involved in apoptosis induced by various stresses [Gil-Gomez et al., 1998; Levkau et al., 1998; Hakem et al., 1999; Hiromura et al., 1999, 2002; Adachi et al., 2001], the elevation of Cdk2 observed in our study represents a slightly different scenario. The UVC-induced elevation of Cdk2 in CHO-K1 cells happens quite *early* following UV irradiation, whereas most stress-induced elevations of Cdk2 occur *later* after treatment as the result of

Fig. 7. Inhibition of PDTC on UV-induced apoptosis in CHO-K1 cells is not due to the antioxidant effect of PDTC. **A**: Measurement of intracellular ROS content with DCF-DA staining analysis. ROS levels were measured at 2 h after treatment with 5 mM PDTC, 5 mM GSH, or at 24 h with 100 μM BSO. ROS content in untreated cells (shaded histogram) was reduced by PDTC and GSH (histogram left-shifted) and was increased by BSO (histogram right-shifted). **B**: Cells after UV irradiation were treated with or without drugs for 24 h and were harvested for measurement of apoptosis by flow cytometric analysis. The sub-G_1 fraction is marked with arrows. Summary of the quantitative analysis of apoptosis are shown as means ± SE of at least three independent experiments. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the degradation of p21 by caspase during apoptosis [Levkau et al., 1998; Adachi et al., 2001].

The results concerning the effect of ROS in UV-induced apoptosis deserve some discussion. We did not find a significant elevation in ROS levels after UV irradiation (data not shown) and our present data do not support the involvement of ROS in UV-induced apoptosis of CHO-K1 cells. Nevertheless, our study might be limited to a certain aspect of anti-oxidation by thiols (GSH), and other endogenous scavengers, such as catalase or superoxide dismutase, were not tested in our study. ROS are a family of oxygen-centered species, including superoxide anions, hydroxyl radicals, singlet oxygen, and hydrogen peroxide. GSH may not be as effective as catalase to eliminate hydrogen peroxide.

In this study, the antioxidant PDTC inhibited UV-induced apoptosis. Although not all antioxidants possess anti-Cdk2 activity, the finding with PDTC raises a concern that the effectiveness of chemotherapy may be compromised by co-administering antioxidant drugs such as PDTC with anticancer drugs such as cisplatin. Our preliminary study indicates that co-treatment of PDTC with cisplatin weakened the cytotoxicity of cisplatin (data not shown).

It is of interest that Cdk4 activity is apparently not involved in the UV-induced apoptosis. Interestingly, levels of cyclin D1 protein are greatly elevated following UV treatment (Fig. 4A). The elevation of cyclin D1 may be indirectly related to the elevation of Cdk2 activity, which in turn releases the E2F activity. It has been shown that cyclin D1 is also among the many targets of E2F [Fan and Bertino, 1997; Inoshita et al., 1999].

Our present study suggests that the UV-induced apoptosis in CHO-K1 cells required Cdk2 and was p53-independent. Apoptosis independent of p53 has been reported in many different systems. For instance, the genotoxins-induced apoptosis occurs without the involvement of p53 in fibroblasts that have a defect in various DNA repair pathways [reviewed by Kaina, 2003]. Besides Cdk2, factors such as interferon regulatory factor-1, c-Jun NH_2-terminal kinase, Ca^{2+}, or p73 have also been shown to be essential for p53-independent apoptosis [e.g., Tamura et al., 1995; Hayashi et al., 2004; Zou et al., 2004; Flinterman et al., 2005]. Regardless of the difference in the signals involved, the p53-independent apoptosis eventually would trigger the activation of endogenous mitochondrial damage pathway and/or the activation of the membrane death-receptors pathway [reviewed by Kaina, 2003].

In summary, deregulation of Cdk2 activity is closely correlated to UV-induced apoptosis in CHO-K1 cells, which do not express $p21^{(waf1/cip1)}$. Our study suggests that modulation of Cdk2 activity may be used as a target for chemotherapy.

REFERENCES

Adachi S, Ito H, Tamamori-Adachi M, Ono Y, Nozato T, Abe S, Ikeda M, Marumo F, Hiroe M. 2001. Cyclin A/cdk2 activation is involved in hypoxia-induced apoptosis in cardiomyocytes. Circ Res 88:408–414.

Bender K, Blattner C, Knebel A, Iordanov M, Herrlich P, Rahmsdorf HJ. 1997. UV-induced signal transduction. J Photochem Photobiol B 37:1–17.

DeGregori J, Leone G, Miron A, Jakoi L, Nevins JR. 1997. Distinct roles for E2F proteins in cell growth control and apoptosis. Proc Natl Acad Sci USA 94:7245–7250.

Dong YB, Yang HL, Elliott MJ, Liu TJ, Stilwell A, Atienza C, Jr., McMasters KM. 1999. Adenovirus-mediated E2F-1 gene transfer efficiently induces apoptosis in melanoma cells. Cancer 86:2021–2033.

Dong YB, Yang HL, Elliott MJ, McMasters KM. 2002. Adenovirus-mediated *E2F-1* gene transfer sensitizes melanoma cells to apoptosis induced by topoisomerase II inhibitors. Cancer Res 62:1776–1783.

Dulic V, Kaufmann WK, Wilson SJ, Tlsty TD, Lees E, Harper JW, Elledge SJ, Reed SI. 1994. p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G_1 arrest. Cell 76:1013–1023.

Elliott MJ, Dong YB, Yang H, McMasters KM. 2001. E2F-1 up-regulates c-Myc and p14(ARF) and induces apoptosis in colon cancer cells. Clin Cancer Res 7:3590–3597.

Ellis RE, Yuan JY, Horvitz HR. 1991. Mechanisms and functions of cell death. Annu Rev Cell Biol 7:663–698.

Fan J, Bertino JR. 1997. Functional roles of E2F in cell cycle regulation. Oncogene 14:1191–1200.

Flinterman M, Guelen L, Ezzati-Nik S, Killick R, Melino G, Tominaga K, Mymryk JS, Gaken J, Tavassoli M. 2005. E1A activates transcription of p73 and Noxa to induce apoptosis. J Biol Chem 280:5945–5959.

Gil-Gomez G, Berns A, Brady HJ. 1998. A link between cell cycle and cell death: Bax and Bcl-2 modulate Cdk2 activation during thymocyte apoptosis. EMBO J 17:7209–7218.

Gu Y, Turck CW, Morgan DO. 1993. Inhibition of CDK2 activity in vivo by an associated 20K regulatory subunit. Nature 366:707–710.

Hakem A, Sasaki T, Kozieradzki I, Penninger JM. 1999. The cyclin-dependent kinase Cdk2 regulates thymocyte apoptosis. J Exp Med 189:957–968.

Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G_1 cyclin-dependent kinases. Cell 75:805–816.

Hayashi Y, Kondo T, Zhao QL, Ogawa R, Cui ZG, Feril LB, Jr., Teranishi H, Kasuya M. 2004. Signal transduction of

p53-independent apoptotic pathway induced by hexavalent chromium in U937 cells. Toxicol Appl Pharmacol 197:96–106.

Hiromura K, Pippin JW, Fero ML, Roberts JM, Shankland SJ. 1999. Modulation of apoptosis by the cyclin-dependent kinase inhibitor p27(Kip1). J Clin Invest 103:597–604.

Hiromura K, Pippin JW, Blonski MJ, Roberts JM, Shankland SJ. 2002. The subcellular localization of cyclin dependent kinase 2 determines the fate of mesangial cells: Role in apoptosis and proliferation. Oncogene 21:1750–1758.

Inoshita S, Terada Y, Nakashima O, Kuwahara M, Sasaki S, Marumo F. 1999. Regulation of the G_1/S transition phase in mesangial cells by E2F1. Kidney Int 56:1238–1241.

Kaina B. 2003. DNA damage-triggered apoptosis: Critical role of DNA repair, double-strand breaks, cell proliferation and signaling. Biochem Pharmacol 66:1547–1554.

Kerr JF, Wyllie AH, Currie AR. 1972. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257.

Kondo S, Barnett GH, Hara H, Morimura T, Takeuchi J. 1995. MDM2 protein confers the resistance of a human glioblastoma cell line to cisplatin-induced apoptosis. Oncogene 10:2001–2006.

Levkau B, Koyama H, Raines EW, Clurman BE, Herren B, Orth K, Roberts JM, Ross R. 1998. Cleavage of p21Cip1/Waf1 and p27Kip1 mediates apoptosis in endothelial cells through activation of Cdk2: Role of a caspase cascade. Mol Cell 1:553–563.

Lin KW, Nam SY, Toh WH, Dulloo I, Sabapathy K. 2004. Multiple stress signals induce p73 beta accumulation. Neoplasia 6:546–557.

Meijer L, Borgne A, Mulner O, Chong JP, Blow JJ, Inagaki N, Inagaki M, Delcros JG, Moulinoux JP. 1997. Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. Eur J Biochem 243:527–536.

Michieli P, Chedid M, Lin D, Pierce JH, Mercer WE, Givol D. 1994. Induction of WAF1/CIP1 by a p53-independent pathway. Cancer Res 54:3391–3395.

Nakanishi M, Adami GR, Robetorye RS, Noda A, Venable SF, Dimitrov D, Pereira-Smith OM, Smith JR. 1995. Exit from G_0 and entry into the cell cycle of cells expressing p21Sdi1 antisense RNA. Proc Natl Acad Sci USA 92:4352–4356.

Schwarz T. 1998. UV light affects cell membrane and cytoplasmic targets. J Photochem Photobiol B 44:91–96.

Tamura T, Ishihara M, Lamphier MS, Tanaka N, Oishi I, Aizawa S, Matsuyama T, Mak TW, Taki S, Taniguchi T. 1995. An IRF-1-dependent pathway of DNA damage-induced apoptosis in mitogen-activated T lymphocytes. Nature 376:596–599.

Tzang BS, Lai YC, Hsu M, Chang HW, Chang CC, Huang PC, Liu YC. 1999a. Function and sequence analyses of tumor suppressor gene *p53* of CHO.K1 cells. DNA Cell Biol 18:315–321.

Tzang BS, Lai YC, Liu YC. 1999b. UV-induced but P53 independent apoptotic death in CHO.K1 cells is promoted by M phase inhibitors. In Vitro Cell Dev Biol Anim 35:17–18.

Tzang BS, Chiang YJ, Lan HC, Liao CB, Liu YC. 2002. Tuning up or down the UV-induced apoptosis in Chinese hamster ovary cells with cell cycle inhibitors. Photochem Photobiol 75:662–667.

Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. 1995. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods 184:39–51.

Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D. 1993. p21 is a universal inhibitor of cyclin kinases. Nature 366:701–704.

Yang Y, Cheng JZ, Singhal SS, Saini M, Pandya U, Awasthi S, Awasthi YC. 2001. Role of glutathione S-transferases in protection against lipid peroxidation. Overexpression of hGSTA2-2 in K562 cells protects against hydrogen peroxide-induced apoptosis and inhibits JNK and caspase 3 activation. J Biol Chem 276:19220–19230.

Zou W, Liu X, Yue P, Zhou Z, Sporn MB, Lotan R, Khuri FR, Sun SY. 2004. c-Jun NH2-terminal kinase-mediated up-regulation of death receptor 5 contributes to induction of apoptosis by the novel synthetic triterpenoid methyl-2-cyano-3,12-dioxooleana-1, 9-dien-28-oate in human lung cancer cells. Cancer Res 64:7570–7578.