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Mechanism of Taxol-Induced Apoptosis in Human SKOV3 Ovarian Carcinoma Cells

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Abstract Taxol is extensively used clinically for chemotherapy of patients with ovarian, breast, and lung cancer. Although taxol induces apoptosis of cancer cells, its exact mechanism of action is not yet known. To determine the mechanism of action of taxol in ovarian cancer, we tested the effects of the drug, on the human ovarian carcinoma cell line, SKOV3. We observed that taxol-induced apoptosis of these cells by phosphatidylserine (PS) externalization and DNA fragmentation. While treatment of cells with taxol resulted in bcl-2 phosphorylation and mitochondrial depolarization, cytochrome c was not released and pro-caspase-3 was not activated. Treatment of SKOV3 cells with taxol, however, resulted in the translocation of AIF from the mitochondria to the nucleus via the cytosol. Taken together, these findings suggest that in SKOV3 cells, taxol induces caspase-independent AIF-dependent apoptosis. J. Cell. Biochem. 91: 1043–1052, 2004.

Key words: SKOV3; apoptosis; taxol; caspase; AIF

Taxol (paclitaxel), a potent drug of natural origin isolated from the bark of the Pacific yew, *Taxus brevifolia* [Wani et al., 1971], is currently used in the treatment of ovarian, lung, and breast cancer. Initial studies on the mechanism of action of taxol have demonstrated that this drug alters microtubule (MT) assembly, by inhibiting MT depolymerization and changing MT dynamics. These effects result in the disruption of the normal reorganization of the MT network required for mitosis and cell proliferation.

Taxol-treated cells are unable to proceed normally through the cell cycle and are arrested in G_2/M phase [Schiff et al., 1979]. Although treatment of various tumor cells with taxol in vivo and in vitro induces apoptosis [Fan, 1999], and taxol has been shown to regulate the expression of several apoptosis-related proteins, including bcl-2, bax, bcl-X, p21-waf, and tumor necrosis factor (TNF)-α [Haldar et al., 1996; Tudor et al., 2000], the exact mechanism by which this drug induces apoptosis is not yet known.

Malfunction of apoptosis has been associated with various diseases, including cancer, autoimmune diseases, neurodegenerative diseases, stroke, cardiac diseases, and bacterial and viral infections [Fadeel et al., 1999a]. Recently two apoptosis pathways have been described, one caspase-dependent and the other caspase-independent [Zamzami and Kroemer, 1999]. Caspases are a family of cystein-dependent aspartate-directed proteases, which have been found to play critical roles in the initiation and execution of apoptosis [Budihardjo et al., 1999]. Following activation of the caspase cascade, downstream molecules are activated, including caspase activated DNase (CAD) and acinus (apoptotic chromatin condensation inducer in the nucleus), leading to

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chromatincondensation and 180-base-pair DNA laddering, a hallmark of apoptotic cell death [Robertson et al., 2000].

More recently, caspase-independent apoptosis has been observed in several cell types [Carmody and Cotter, 2000; Dumont et al., 2000; Braun et al., 2001; Loeffler et al., 2001; Marzo et al., 2001]. One of the proteins responsible for caspase-independent chromatin condensation is apoptosis-inducing factor (AIF) [Susin et al., 1999]. AIF (57 kDa) is a phylogenetically ancient conserved flavoprotein that is confined to the mitochondrial intermembrane space in healthy cells. Upon lethal signaling, AIF translocates from the mitochondria to the nucleus, via the cytosol. Although this protein possesses the ability to induce caspase-independent peripheral chromatin condensation and large-scale DNA fragmentation, the molecular signaling mechanism currently remains unclear [Cande et al., 2002a]. Mouse AIF (612 amino acids) and its human counterpart (613 amino acids) [Susin et al., 1999] are highly homologous, with 92% overall identity. The *AIF* gene is localized within the mouse X chromosome region, A6, which is syntenic to the human X chromosome region, Xq25-26 [Daugas et al., 2000a].

AIF is neutralized by heat-shock protein (HSP)70, in a reaction that appears to be independent of ATP and the ATP-binding domain (ABD) of HSP70. The mechanism of action of this protein is thus distinct from the previously described Apaf-1/HSP70 interactions which require both ATP and HSP70 ABD. Intriguingly, HSP70 lacking ABD (HSP70ΔABD) inhibits apoptosis induced by serum withdrawal, staurosporine, and menadione, three models of apoptosis that are additionally affected by micro-injection of anti-AIF antibody or genetic ablation of AIF [Cande et al., 2002a]. Collectively, recent studies suggest that AIF plays a role in the regulation of caspase-independent cell death.

Numerous investigations on the mechanism of apoptosis report that this type of cell death is induced via the caspase-dependent pathway. In contrast, our data show that taxol-induced apoptosis is caspase-independent. In this report, we investigate the mechanism by which taxol induces apoptosis. We recently demonstrated that AIF is involved in taxol-induced apoptosis in the human ovarian carcinoma cell line, SKOV3.

MATERIALS AND METHODS

Materials

Taxol was obtained from Bristol–Myers Squibb Pharmaceutical Group (Montreal, QC, Canada), dissolved in DMSO and stored at room temperature in the dark. Propidium iodide (PI) was purchased from Sigma (St. Louis, MO) and Annexin V-FITC Apoptosis detection kit was from BD BioSciences (San Diego, CA). Alexa Flour 488 dye (FITC) and 5,5′-6,6′-tetrachloro-1,1′-3,3′-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1) were obtained from Molecular Probes (Eugene, OR). Mouse monoclonal antibodies to bcl-2 and pro-caspase-3, rabbit polyclonal antibody to AIF, and secondary antibodies including horse-radish peroxidase (HRP)-conjugated goat anti-mouse IgG, were from Upstate (Lake Placid, NY). Cytochrome c (A-8) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and biotin conjugated goat anti-rabbit and mouse IgG were from ZYMED Laboratories, Inc. (San Francisco, CA). Polyvinylidene difluoride (PVDF) membrane was obtained from Amersham Pharmingen Biotech. (Piscataway, NJ), and enhanced chemiluminescence reagent was from Pierce (Rockford, IL).

Cell Culture

The human ovarian carcinoma cell line SKOV3 (HTB-77) was purchased from American Tissue Culture Collection (ATCC, Manassas, VA), and cells were grown at 37°C in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin (pH 7.4) in a humidified atmosphere containing 5% CO_2. All cell culture reagents were purchased from Gibco BRL (Grand lsland, NY). Through all the experiments, SKOV3 cells were treated with taxol for 24 h.

Determination of Cell Cycle and DNA Fragmentation Rate

To measure the amount of DNA fragmentation and the cell cycle, cells were harvested, resuspended at 1×10^6 cells/ml, washed with PBS, and fixed in ice-cold 70% ethanol for 1 h at 4°C. The cells were subsequently centrifuged, resuspended, incubated for 30 min in PBS containing 0.5 mg/ml RNase A and 40 μg/ml PI at 37°C, and analyzed with a Becton Dickson (San Jose, CA) FACSCalibur flow cytometer [Ormerod et al., 1992].

Measurement of Phosphatidylserine (PS) Externalization and Chromatin Condensation

PS externalization was assayed by two-color analysis of FITC-labeled annexin V staining of 1×10^6 cells/ml, together with PI staining of 5×10^3 cells/ml, followed by flow cytometry and Leica TCS SP2 confocal microscopy (Leica, Germany). Data acquisition and analysis were performed using the CellQuest program (Becton Dickson). Positioning of quadrants on annexin V/PI dot plots was performed as described previously [Van Engeland et al., 1996]. This method was able to distinguish among living (annexin V^-/PI^-), early apoptotic (annexin V^+/PI^-), late apoptotic (annexin V^+/PI^+), and necrotic (annexin V^-/PI^+) cells [Pietra et al., 2001].

Detection of Mitochondrial Membrane Potential ($\Delta\Psi m$)

The mitochondrial membrane potential was analyzed using JC-1, a lipophilic cationic fluorescent dye capable of selectively entering mitochondria and acting as a dual emission probe that reversibly changes color from green (FL-1) to greenish orange (FL-2) as the mitochondrial membrane becomes more polarized [Cossarizza et al., 1993]. Cells, 1×10^6 and 5×10^3, respectively, were incubated with 5 μg/ml JC-1 (made up as a 5 mg/ml stock in DMSO) for 30 min at 37°C in darkness. Cells were washed with PBS at 4°C, and analyzed by flow cytometry and confocal microscopy.

Western Blot Analysis

Control and taxol-treated cells were scrapped and washed with ice-cold PBS. Cells (2×10^6 cells) were lysed with 100 μl lysis buffer (50 mM Tris, 150 mM NaCl, 10% SDS, 1% NP-40, 1% Triton X-100, 1 mM EGTA, 1 mM PMSF, 1 μM pepstain, 1 μM leupeptin, 0.3 μM aprotinin). After 1 h incubation on ice, the lysates were centrifuged at 15,000*g* for 15 min at 4°C, and the protein content in the supernatant was determined using Bio-Rad Protein Assay Dye Reagent. Each lysate was dissolved in sample buffer (60 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 140 mM mercaptoethanol, 0.002% bromophenol blue), and the samples were boiled for 5 min and subjected to electrophoresis on an SDS–polyacrylamide gel (100 μg protein/lane). Proteins were electrotransferred onto PVDF membranes, which were immunoblotted with anti-pro-caspase-3 (1:1,000 dilution) or anti-bcl-2 (1:1,000 dilution) antibodies. Antibodies were detected with the appropriate HRP-conjugated secondary antibody (1:5,000 dilution) and enhanced chemiluminescence reagent.

Determination of H_2O_2 Generation

For detection of H_2O_2 generation, cells were harvested at 1×10^6 cells/ml, washed in PBS, resuspended, and incubated for 30 min at room temperature in Mixing dye (0.5 ml DMSO, 2% pluronic F127 (Molecular Probes) containing 10 μM H_2DCFDA (Molecular Probes). The cells were washed with PBS and analyzed by flow cytometry.

Immunofluorescence Assay

The translocation of AIF in taxol-treated SKOV3 cells was determined by confocal microscopy. Cells were grown on coverslips, washed with PBS, fixed with 4% paraformaldehyde in PBS at room temperature for 1 h, and permeabilized with 0.3% Triton X-100 for 30 min at room temperature. The cells were incubated with rabbit anti-AIF (0.5 μg/ml) for 12 h at 4°C, washed three times with PBS, and incubated with biotin-conjugated secondary antibodies (2 μg/ml) for 4 h at 4°C. The cells were incubated with Alexa Flour 488 dye (1:500 dilution) for 1 h at room temperature, washed three times with PBS, mounted with DAKO fluorescent mounting medium (DAKO, Carpinteria, CA), and observed by confocal microscopy.

RESULTS

Cell Cycle Arrest and DNA Fragmentation

The effects of taxol on polymerization of the microtubule and stimulation of microtubule bundle formation, which blocks entry into the S phase, are well known. Inhibition of S phase entry leads to blockage of cell proliferation and induction of necrosis [Yeung et al., 1999]. To investigate the type of cell death induced by taxol, PS externalization, and PI uptake in SKOV3 cells were analyzed. Treatment with 5 μM taxol increased the number of primary apoptotic cells (annexin V^+/PI^-) 5-fold and the number of secondary necrotic cells (late apoptosis, annexin V^+/PI^+) 2-fold compared with control, untreated SKOV3 cells (taxol-treated, $26.44 \pm 1.96\%$; control, $5.24 \pm 1.24\%$, and taxol-treated, $5.01 \pm 0.34\%$; control, $2.34 \pm 0.64\%$, respectively; Fig. 1a). Taxol treatment also increased PI uptake, suggesting that this drug

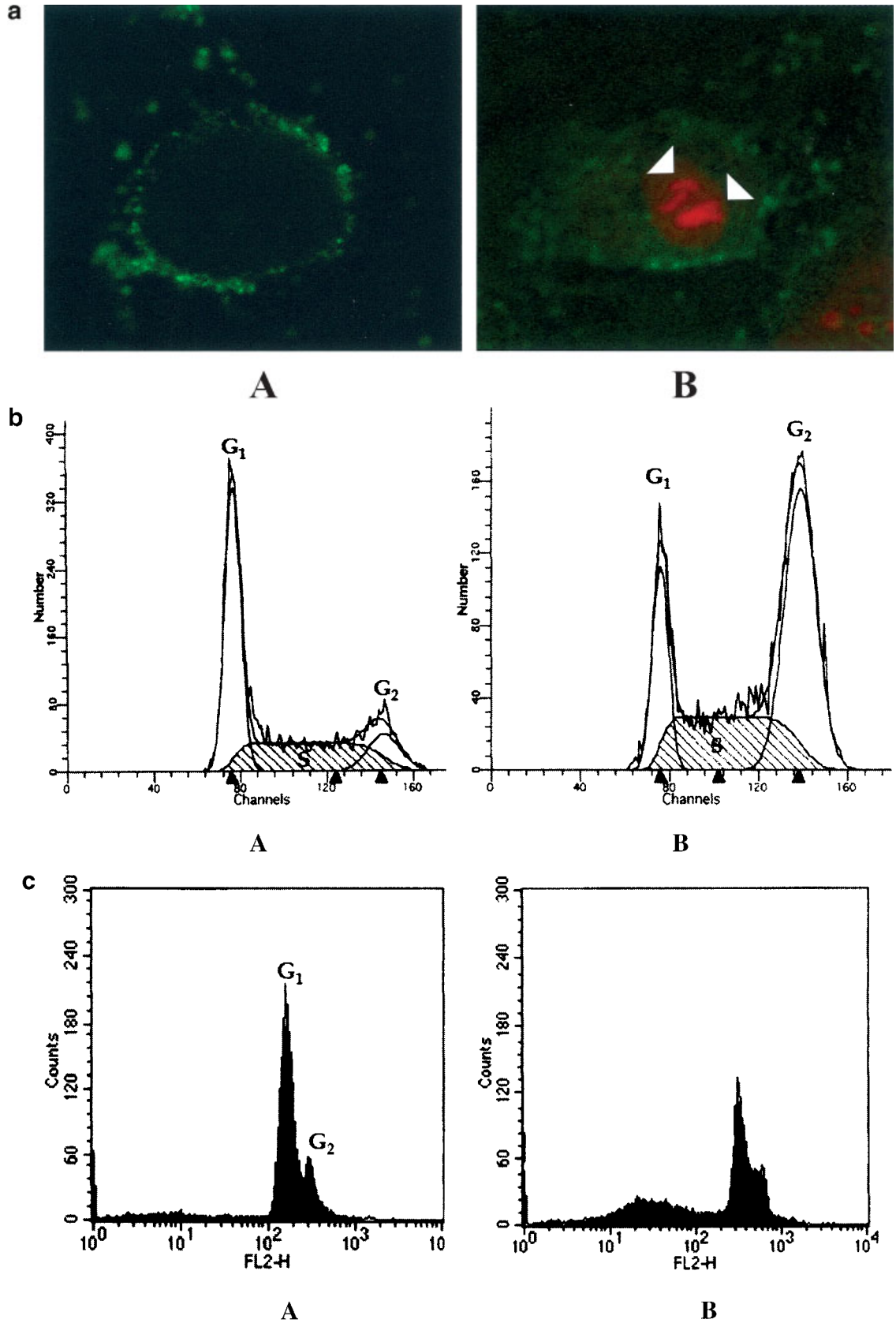

Fig. 1.

induced chromatin condensation. To characterize the effect of taxol-induced DNA damage, SKOV3 cells were cultured in taxol, stained with PI, and assayed by flow cytometry. Compared with control cells, taxol-treated cells progressed to G_2/M phase arrest after 24 h (Fig. 1b), and there was a 2-fold increase in the number of cells in sub G_1 phase (taxol-treated, $11.33 \pm 1.91\%$; control, $5.25 \pm 1.23\%$, Fig. 1c). Notably, DNA from the Sub G_1 phase is less than diploid (the DNA is broken and fragmented). Our results show that taxol induces the early and late stages of apoptosis and not necrosis.

Taxol Does not Induce Caspase-3 Activation

The caspase family of proteins is important in apoptosis [Zamzami and Kroemer, 1999]. A number of reports show that these proteins are activated by drugs that display an apoptosis-inducing effect. Therefore, we attempted to determine whether taxol induces apoptosis through caspase-dependent or -independent mechanism in SKOV3 cells. By Western blot analysis, we found that taxol, at concentrations of 2 and 5 μM, induced bcl-2 phosphorylation, but did not activate caspase-3 (Fig. 2). These findings suggest that, in SKOV3 cells, taxol induces apoptosis through a caspase-independent pathway.

Taxol Induces Mitochondrial Depolarization

Mitochondrial membrane depolarization is an early event of apoptosis, increasing mitochondrial membrane permeability (MMP) and facilitating the release of pro-apoptotic factors, including cytochrome c, AIF and other, as yet unidentified, factors into the cytosol [Cande et al., 2002b; Ravagnan et al., 2002]. Using the mitochondria-specific probe, JC-1, a lipophilic cationic fluorescent dye with dual emission wavelengths, we tested the effect of taxol on mitochondrial depolarization in SKOV3 cells. Increased mitochondrial membrane potential has been shown to increase JC-1 fluorescence at 530 nm (FL-1H), corresponding to its monomeric form, and to reduce JC-1 fluorescence at 590 nm, corresponding to its dimeric form. Taxol treatment increased fluorescence at 530 nm from 2.77 ± 0.90 (mean $\pm$ SD) in normal cells to 4.51 ± 0.83 ($P < 0.05$) after 24 h in culture (Fig. 3a). By confocal microscopy, taxol was also found to increase the mitochondrial membrane potential (Fig. 3b).

Reactive Oxygen Species (ROS) Is not Generated by Taxol Treatment

ROS are among the molecular components that can be detected during the process of apoptotic cell death. ROS can be generated from several intracellular locations, including the microsomal pathway and the mitochondrial electron transport chain [Kukielka et al., 1994; Thannickal and Fanburg, 1995]. Following treatment with 5 μM taxol, however, the rate of ROS generation (3.11 ± 0.31) was not that different from the rate observed in normal cells (3.22 ± 0.39) (Fig. 4).

Effect of Taxol on AIF Translocation Into Nucleus

The AIF protein is a caspase-independent pro-apoptotic factor released from mitochondria and translocated into the nucleus [Susin et al., 1999]. The connection between AIF and mitochondrial membrane potential in caspase-independent apoptosis is not yet clear. Confocal microscopy of cells stained with anti-AIF antibody (green fluorescence of FITC) was used to test the effect of taxol on AIF translocation. In untreated cells, spots of green fluorescence indicated that AIF was present in the cytosol, presumably in the mitochondria (Fig. 5). In taxol-treated cells, the fluorescence was located in the nucleus, indicating that AIF had been translocated (Fig. 5).

Fig. 1. **a**: Taxol-induced early- and late-stage apoptosis in SKOV3 carcinoma cells. SKOV3 cells were treated with 5 μM taxol for 18 h (**A**) and 24 h (**B**). Cells were assayed by two-color analysis involving FITC-labeled annexin V and propidium iodide (PI) staining, followed by flow cytometry and Leica TCS SP2 confocal microscopy (Leica, Germany). A: Early-stage apoptotic cells were stained with annexin V (conjugated FITC) only. B: Late-stage apoptotic cells were stained with both annexin V and PI, due to dysfunctional plasma membrane fluidity. **b**: The effect of taxol on SKOV3 distribution in distinct cell cycle phases, as determined by flow cytometry. **A**: Untreated cells. **B**: Cells treated with 5 μM taxol for 24 h. Cells were stained with PI and analyzed by flow cytometry. The majority of cells were arrested at the G_2/M phase after taxol treatment. **c**: Assessment of taxol-induced apoptosis by measurement of hypodiploid DNA content in SKOV3 carcinoma cells. **A**: Untreated cells. **B**: Cells treated with 5 μM taxol for 24 h. Cells were analyzed by PI staining to determine the hypodiploid DNA (fragmented DNA) proportion. Data acquisition and analysis were performed on a FACScalibur flow cytometer using the CellQuest program (Becton Dickson). The sub G_1 percentage signifies the cell proportion of apoptosis procedure. Apoptosis of taxol-treated cells was increased 2-fold ($11.33 \pm 1.91\%$), in comparison to the control ($5.25 \pm 1.23\%$). [†]mean $\pm$ SE of three independent experiments ($P < 0.05$).

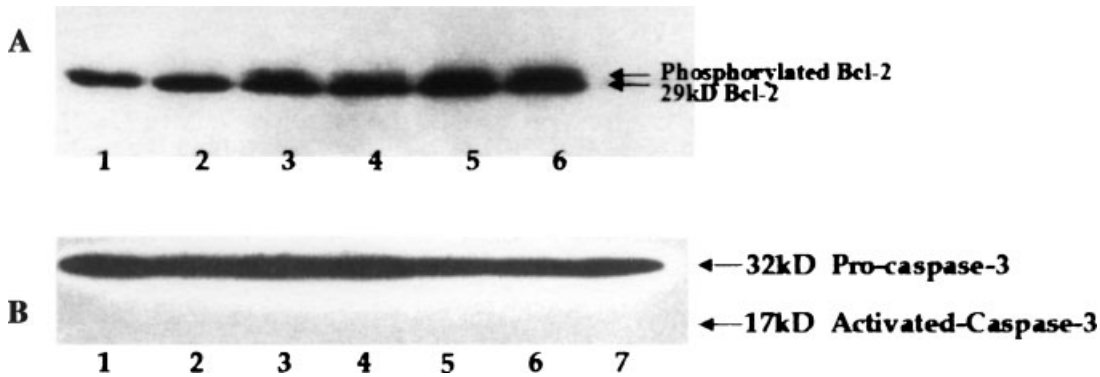

Fig. 2. Taxol induces phosphorylation of bcl-2, but not activation of caspase-3 in SKOV3 cells. SKOV3 cells were treated with 2 and 5 μM taxol for 24 h, and analyzed by Western blotting. **A**: Phosphorylation of bcl-2. **Lanes 1** and **2**, control; **lanes 3** and **4**, cells treated with 2 μM taxol; **lanes 5** and **6**, cells treated with 5 μM taxol. **B**: Induction of caspase-3 protein processing. Lanes 1, 2, and 7, control; lanes 3 and 4, treatment with 2 μM taxol; lanes 5 and 6, treatment with 5 μM taxol.

These findings thus suggest that the mitochondria play a role in triggering a caspase-independent apoptotic pathway through loss of mitochondrial membrane potential and the release of AIF protein in taxol-treated SKOV3 cells.

DISCUSSION

Although taxol has been shown to induce apoptosis in a variety of cell types, its mechanism of action is not fully known. We have shown here that this drug has an unusual mode of action in human ovarian carcinoma cells, a mechanism that does not require caspase-3 activation [Ofir et al., 2002]. The effect of taxol on caspase-3 activation is cell type specific, since this drug has been shown to induce caspase-3 enzymatic activity in other human carcinoma cell lines, including the acute myelocytic leukemia cell line HL-60, as well as lung and gastric cancer cell lines [Perkins et al., 1998; Goncalves et al., 2000; Weigel et al., 2000]. These and other results [Blagosklonny et al., 1996a,b; Salah-Eldin et al., 2003] support the notion that the same apoptotic stimulant may utilize different apoptotic pathways in different cell types.

Taxol treatment induces a biphasic decrease of viable cells in various breast cancer cell lines. In the lower concentration range (0.005–0.05 μM), taxol stabilizes the spindle during mitosis, thereby blocking cell division. This mitotic block leads to inhibition of cell proliferation and apoptosis induction. In the higher concentration range (5–50 μM), taxol mainly increases polymerization of the microtubule and stimulates the formation of microtubule bundles, which block entry into the S phase. This inhibition of S phase entry results in blockage of cell proliferation and induction of necrosis [Yeung et al., 1999]. Taxol-induced apoptosis is additionally linked to mitosis inhibition. Prevention of polymerization or depolymerization of cellular microtubules by cancer therapeutic drugs induces phosphorylation of bcl-2, thus abrogating the normal anti–apoptotic function of the protein and initiating the apoptotic program in differentiating cancer cells [Haldar et al., 1997]. In our study, SKOV3 treated 5 μM taxol displayed cell arrest. Interestingly, in contrast to the breast cancer cell lines, i.e., BT20, BT474, MDA, MCF-7, and SKRB3, studied earlier [Yeung et al., 1999], taxol-induced apoptotic death (see Fig. 1a) in the SKOV3 cell line, and not necrosis, at the concentration of 5 μM.

In contrast, TNF-α has been shown to activate two apoptotic signaling cascades, one dependent on ROS and the other independent of ROS, both of which converge on caspase activity [Sidoti-de Fraisse et al., 1998]. In addition, superoxides and lipid peroxidation are increased during apoptosis induced by many stimuli [Ravagnan et al., 2002]. We have shown here that treatment of SKOV3 cells with taxol did not generate ROS, including H_2O_2, suggesting that this drug induces ROS-independent apoptosis. This finding is in agreement with previous results [Lin et al., 2000].

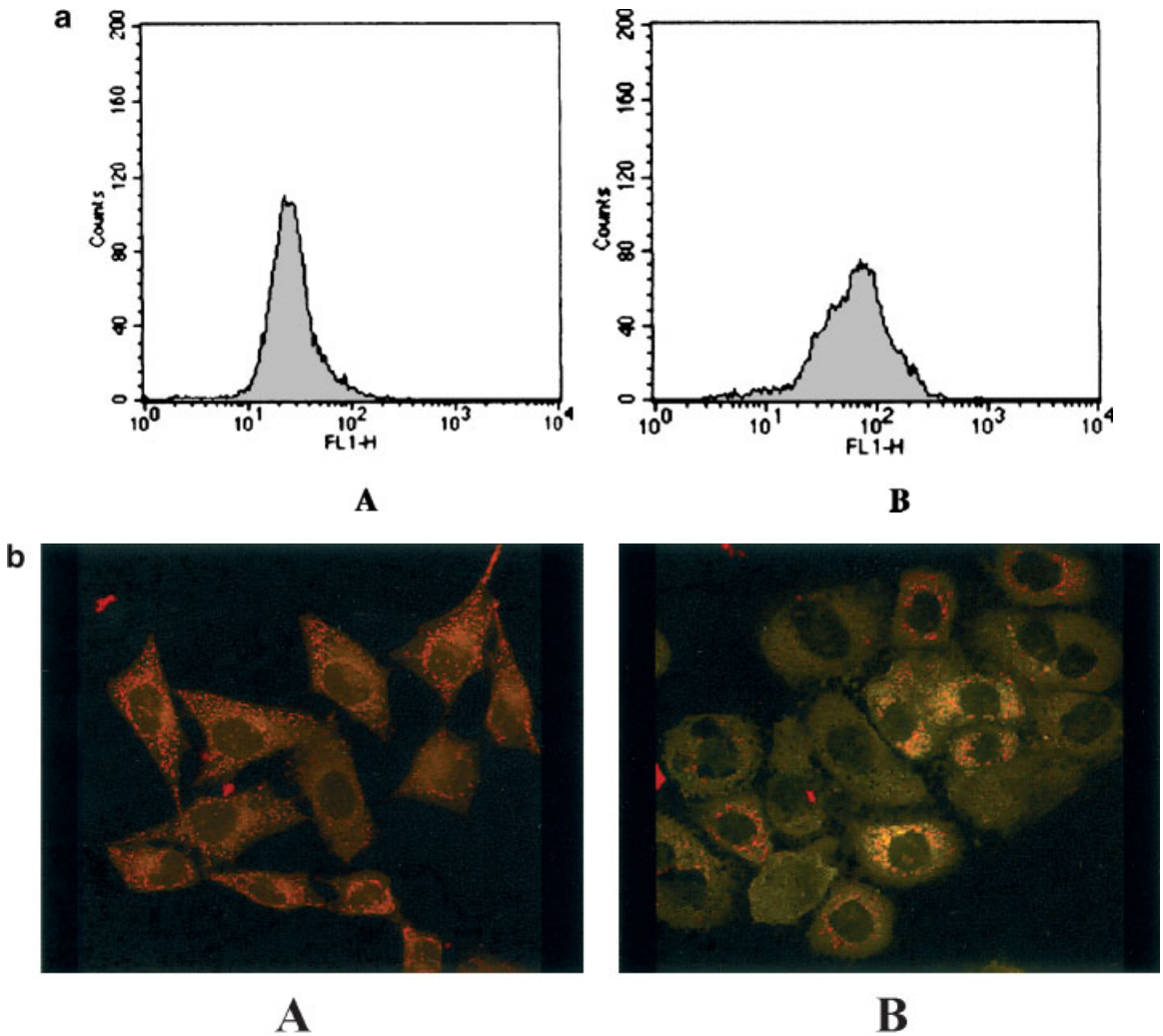

Fig. 3. **a**: Taxol induces mitochondrial depolarization, as determined by flowcytometry (Green color Emission Shifting). Taxol-treated SKOV3 cells were incubated with JC-1 dye (5 μg/ml) for 30 min at 37°C, and fluorescence intensities were measured. **A**: Untreated cells. **B**: Cells treated with 5 μM taxol for 24 h. Relative intensity of emission at 530 nm was increased from 2.77 ± 0.90 (mean ± SD) for normal cells to 4.51 ± 0.83 ($P < 0.05$) for taxol-treated cells within 24 h. **b**: Staining with JC-1 revealed a decrease in mitochondrial membrane potential (MMP, $\Delta\Phi m$) upon taxol treatment. Taxol-treated SKOV3 cells were incubated with JC-1 dye (5 μg/ml) for 30 min at 37°C, followed by Leica TCS SP2 confocal microscopy (Leica). **A**: Untreated cells. **B**: Cells treated with 5 μM taxol for 24 h.

In recent years, numerous studies have demonstrated that most, if not all, cells experience a collapse of their mitochondrial membranes as a prelude to nuclear DNA degradation and apoptosis [Ravagnan et al., 2002]. In agreement with this, we have shown here that, in SKOV3 cells, taxol decreases mitochondrial membrane potential. Indeed, mitochondria are now thought to act as key coordinators of apoptosis [Green and Reed, 1998]. Several pro-apoptotic signaling and damage pathways, linked to the bcl-2 family of proteins, converge on mitochondria to induce mitochondrial membrane permeabilization (MMP) [Fadeel B and Zhivotovsky, 1999b]. While the inner mitochondrial membrane (IMM) remains relatively intact, the outer mitochondrial membrane (OMM) becomes completely permeabilized to proteins, resulting in leakage from the mitochondrial intermembrane space of proteins such as pro-caspases 2, 3, and 9, cytochrome c, AIF, Endo G (Endonuclease G), and heat shock proteins (Hsp) 10 and 60 [Ravagnan et al., 2002]. Emerging evidence suggests that translocation of mitochondrial AIF into the cytosol and then into the nucleus is a hallmark of

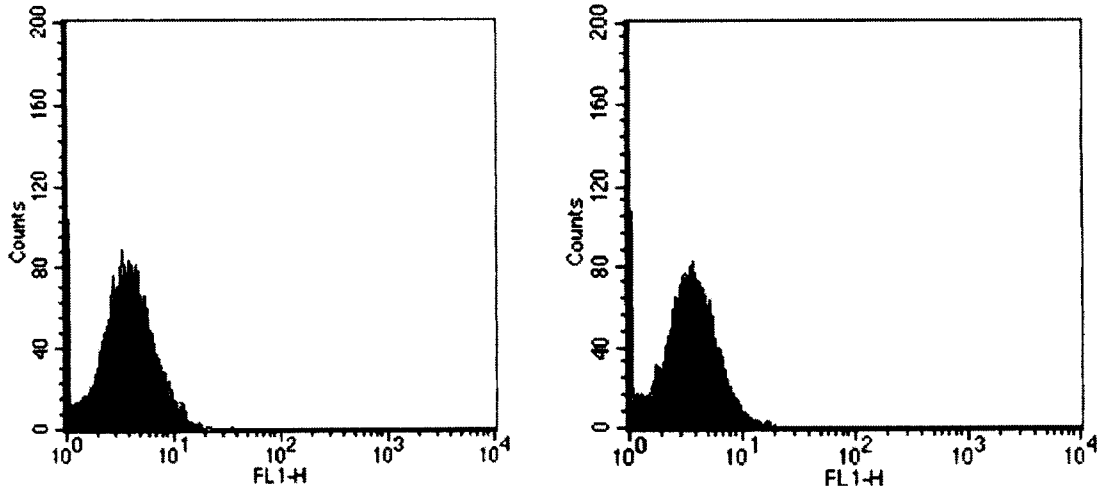

Fig. 4. A H_2DCFDA assay reveals that taxol has no effect on intracellular peroxide levels. **A**: Untreated cells. **B**: Taxol-treated SKOV3 cells (5 μM, 24 h) were incubated for 30 min at room temperature in Mixing dye (0.5 ml DMSO, 2% pluronic F127 (Molecular Probes) containing 10 μM H_2DCFDA (Molecular Probes). Cells were analyzed by flow cytometry. No significant differences in ROS generation were observed between taxol-treated cells ($3.11 \pm 0.31\%$) and normal cells ($3.22 \pm 0.39\%$). †mean ± SE of three independent experiments ($P > 0.05$).

caspase-independent apoptosis [Cande et al., 2002a; Shih et al., 2003]. In support of this, we have shown here that, in SKOV3 cells, taxol treatment induces translocation of AIF after 24 h, providing further evidence that taxol induces caspase-independent apoptosis in SKOV3 cells.

Over-expression of the anti-apoptotic protein, bcl-2, has been found to attenuate AIF redistribution in mammalian cell lines [Daugas et al., 2000b]. Our finding, that bcl-2 was phosphorylated in SKOV3 cells, suggests that, in this cell line, phosphorylated bcl-2 had lost its ability to suppress taxol-induced apoptosis [Haldar et al., 1996].

AIF is a phylogenetically conserved mitochondrial intermembrane flavoprotein that has the ability to induce apoptosis via a caspase-

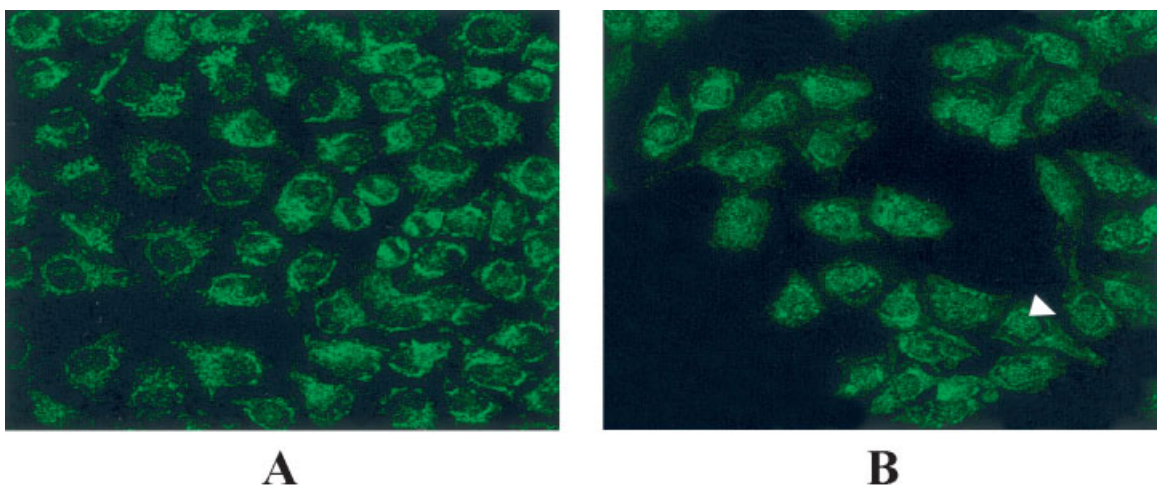

Fig. 5. Translocation of the apoptosis-inducing factor (AIF) into the nucleus induced by taxol. Taxol-treated (5 μM, 24 h) and control cells were fixed and incubated with rabbit anti-AIF (0.5 μg/ml) for 12 h at 4°C, followed by incubation with biotin-conjugated secondary antibodies (2 μg/ml) for 4 h at 4°C. Cells were treated with Alexa Flour 488 dye (1:500 dilution) for 1 h at room temperature, mounted with DAKO fluorescent mounting medium (DAKO), and analyzed by confocal microscopy. **A**: Control, **B**: Taxol (5 μM). In taxol-treated cells, AIF localizes to the cytosol and nucleus.

independent pathway. AIF plays an important role in inducing nuclear chromatin condensation as well as large-scale DNA fragmentation (approximately 50 kb), and is essential for programmed cell death. Microinjection of recombinant AIF leads to the release of AIF-GFP and Cyt-c-GFP, indicating that ectopic AIF favors permeabilization of the outer mitochondrial membrane. These mitochondrial effects of AIF are caspase-independent. Upon prolonged culture, transfection-induced overexpression of AIF results in spontaneous translocation of AIF-GFP from mitochondria, nuclear chromatin condensation, and cell death [Loeffler et al., 2001].

However, in these cells, the mechanism of apoptosis may be not solely dependent on AIF. This is because, although the majority of AIF is translocated into the nucleus, a proportion remains in the cytosol prior to apoptosis by taxol treatment, whereas most of the protein translocates to the nucleus following treatment with selenium (data not shown). Our results show that treatment with taxol induces bcl-2 phosphorylation, which is generally believed to promote paclitaxel-initiated apoptosis [Haldar et al., 1995, 1998; Blagosklonny et al., 1997; Roth et al., 1998].

Another recently isolated mitochondrial factor, Endo G, has been shown to translocate from the mitochondria to the nucleus once apoptosis is induced [Li et al., 2001]. Nuclear Endo G can sequentially catalyze both high molecular weight DNA cleavage and oligonucleosomal DNA breakdown in a caspase-independent manner [Widlak et al., 2001]. The relationship between Endo G and AIF, however, is not yet known. Therefore, further experiments are necessary to investigate the effects of taxol on Endo G translocation, and the link between AIF and Endo G.

In summary, we have shown that, in the human ovarian carcinoma cell line, SKOV3, taxol induces caspase-independent apoptosis through the collapse of mitochondrial membrane potential and the translocation of AIF into the nucleus.

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