Epothilones induce human colon cancer SW620 cell apoptosis via the tubulin polymerization–independent activation of the nuclear factor-κB/IκB kinase signal pathway

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Abstract

Molecular mechanisms underlying epothilone-induced apoptotic cell death were investigated in SW620 human colon cancer cells. Treatment with epothilone B and D at different concentrations (1–100 nmol/L) dose-dependently inhibited cell growth and caused cell cycle arrest at G_2-M, which was followed by apoptosis. Consistent with this induction of apoptotic cell death, epothilone B and D enhanced the constitutional activation of nuclear factor-κB (NF-κB) via IκB degradation through IκB kinase (IKKα and IKKβ) activation, and this resulted in p50 and p65 translocation to the nucleus. Moreover, cells treated with sodium salicylic acid, an IKK inhibitor, or transiently transfected with mutant IKKα and β did not show epothilone-induced cell growth inhibition or p50 translocation, although p65 was still translocated to the nucleus. Treatment with epothilone B and D also enhanced β-tubulin polymerization and the formation of p50/β-tubulin complex. However, β-tubulin polymerization was not inhibited in the cells treated by sodium salicylic acid or transiently transfected with mutant IKKα and β. Moreover, epothilone B and D increased the expressions of NF-κB–dependent apoptotic cell death regulatory genes, i.e., Bax, p53, and the active form of caspase-3, but reduced Bcl-2 expression, and these actions were partially reversed by salicylic acid. In addition, caspase-3 inhibitor reduced epothilone B–induced cell death and NF-κB activation. These findings suggest that the activation of NF-κB/IKK signals plays an important role in the epothilone-induced apoptotic cell death of SW620 colon cancer cells in a tubulin polymerization–independent manner. [Mol Cancer Ther 2007;6(10):2786–97]

Introduction

Drugs that target microtubules are the most commonly prescribed anticancer drugs (1). A macrocyclic polyketide class of compounds known as the epothilones has generated substantial interest over the last few years in the areas of chemistry, biology, and medicine due to the strong inhibitory effect shown by family members on the growths of numerous cancer cells (2, 3).

Epothilones are novel nontaxane microtubule-targeting agents that induce microtubulin polymerization and stabilization and are produced by the myxobacteria *Sorangium cellulosum*. Epothilones have been reported to trigger apoptotic cell death in several cancer cell lines *in vitro* and to inhibit tumor cell growth *in vivo* (2, 3). Like paclitaxel (Taxol), epothilones have been reported to block the cell cycle at G_2-M and to subsequently induce apoptotic cell death in several cancer cells (2, 4). However, the biochemical mechanisms that underlie epothilone-induced cancer cell growth inhibition are not well understood.

Microtubules, the cytoskeletal structures formed by the polymerization of tubulin heterodimers, play a crucial role in many biological processes, including mitosis, cell-cell communication, intracellular transport, cell growth, and apoptotic cell death (5, 6). Moreover, it has been reported that microtubule disruption by colchicines, *Vinca* alkaloids, or nocodazole (depolymerization agents), or by paclitaxel, epothilone, or laulimalide (stabilization agents) modulate several gene expressions and the activities of protein kinases involved in cell cycle arrest and apoptosis (7, 8). These protein kinases include Bcl-2, Cdc25C, survivin, myc, caspase, $p34^{cdc2}$, Ras/Raf, protein kinase C/protein kinase A, and c-Jun NH_2-terminal kinase in addition to nuclear factor-κB (NF-κB) (9, 10).

Accumulating evidence indicates that the activation of NF-κB plays an important role in coordinating the control

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of apoptosis (11, 12). Although NF-κB activation generally inhibits apoptosis, it has been reported that microtubule-disrupting agents induce cancer cell apoptosis by activating NF-κB (13–16). In a series of studies on paclitaxel, it was found that paclitaxel significantly down-regulates IκBα?, and that this in turn promotes NF-κB nuclear translocation of its DNA-binding activity, which eventually results in a marked increase in sensitivity to paclitaxel-induced apoptotic cell death (14–16). In contrast, glucocorticoids were found to antagonize paclitaxel-mediated NF-κB nuclear translocation and activation via IκBα protein synthesis (13). Furthermore, it has been reported that paclitaxel and *Vinca* alkaloid activate IκB kinase (IKK) activity during cancer cell apoptosis (17), which suggests that the activation of the NF-κB/IKKs signal may contribute to the microtubule-disrupting agent–induced apoptosis of solid tumor cells. Rearrangement of the β-tubulin cytoskeleton by microtubule-disrupting agents has been suggested as a mechanism underlying the nuclear translocation and activation of NF-κB (9, 10, 18). However, it is not clear whether NF-κB activation is a prerequisite for the induction of apoptosis; nor is it clear whether there is a relationship between microtubule polymerization disruption and NF-κB activation during microtubule-disrupting agent–induced cancer cell apoptosis.

In this study, we investigated whether epothilones activate NF-κB and IKKs during epothilone-induced apoptotic cell death and attempted to clarify the relationship between microtubule polymerization by epothilone and the activation of NF-κB/IKKs in SW620 human colon cancer cells.

Materials and Methods

Chemicals

Epothilones (Fig. 1) were synthesized as previously described (19) and were dissolved in DMSO. DMEM, penicillin, streptomycin, and fetal bovine serum (FBS) were purchased from Life Technologies. Taxol and pifithrin-α, a p53 inhibitor, were purchased from Sigma Aldrich. Caspase-3 inhibitor II (z-DEVD-fmk) and caspase-9 inhibitor I (z-LEHD-fmk) were from Calbiochem-Novabiochem, Inc. Membrane-permeable Bax inhibitor peptide P5 (Pro-Met-Leu-Lys-Glu) and negative control (Ile-Pro-Met-Ile-Lys) were from Tocris.

Cell Culture

SW620 human colon cells were obtained from the American Type Culture Collection (Cryosite) and were grown in RPMI 1640 containing 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C in 5% CO_2 humidified air.

Cell Viability Assay

Cytotoxic effects were evaluated in the cells cultured for 12, 24, 36, 48, and 72 h using Dojindo's tetrazolium salt, WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt) cell

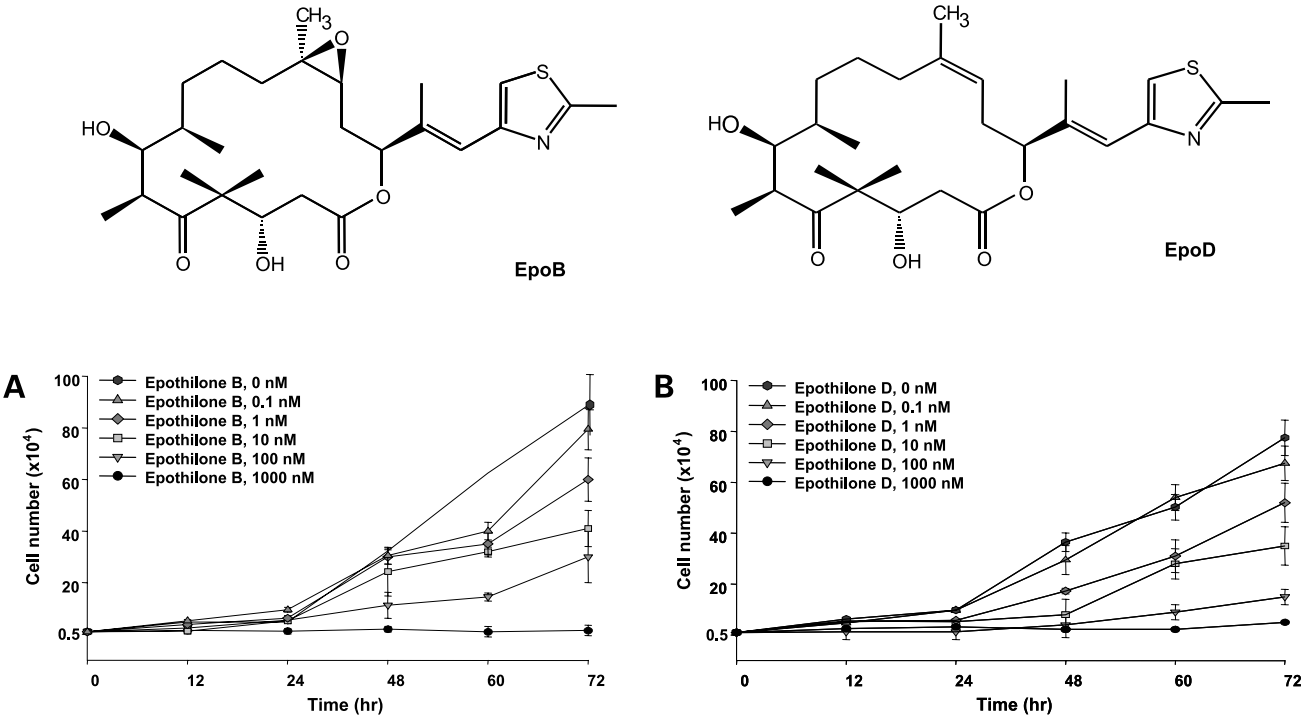

Figure 1. Structures of epothilones and cell viabilities of SW620 human colon cancer cells after epothilone treatment. SW620 cells were treated with epothilone B or D at concentrations ranging from 0 to 1000 nmol/L for up to 72 h. Cell viabilities were determined by direct cell number counting (**A** and **B**) as described in Materials and Methods. *Points,* means of three experiments done in triplicate; *bars,* SD.

counting kits (Dojindo Laboratory). Briefly, cells were plated on 96-well plates at 5×10^3 cells per well. The cells were then incubated at 37°C in 5% CO_2 for 6 h and then treated with epothilone B or D (0, 10, 100, or 1000 nmol/L). After incubation for 12, 24, 36, 48, or 72 h, 10 μL per well of the cell proliferation reagent WST-8 was added to each well. Cells were then incubated for an additional 4 h in a humidified atmosphere. Metabolic activity was quantified by measuring absorbance at 450 nm using a microplate reader.

Western Blot Analysis

Western blot analysis was done as described previously (20). The protein-transferred membranes were incubated for 5 h at room temperature with specific antibodies: rabbit polyclonal antibodies against p65 (1:500) and goat polyclonal antibody for p50 (1:500) or mouse monoclonal IKKα and β antibody (1:500; Santa Cruz Biotechnology Inc.). The blot was then incubated with corresponding conjugated anti-rabbit immunoglobulin G (IgG)–horseradish peroxidases (1: 1,000; Santa Cruz Biotechnology Inc.). Immunoreactive proteins were detected using the ECL Western blotting detection system. The relative densities of protein bands were determined by densitometry using MyImage (SLB) and quantified using Labworks 4.0 (UVP Inc.).

Gel Electromobility Shift Assay

The DNA binding activity of NF-κB was determined using an electrophoretic mobility shift assay (EMSA) done according to the manufacturer's recommendations (Promega). In short, SW620 cells were cultured on 100-mm culture dishes. After treatment with epothilone B and D (0, 10, 100, and 1000 nmol/L) or vehicle for 24 h, the cells were washed twice with PBS, followed by the addition of 1 mL of PBS, and the cells were scraped into a cold Eppendorf tube. Nuclear extracts were prepared and processed for EMSA as previously described (20). The relative densities of the DNA-protein binding bands were scanned by densitometry using MyImage (SLB), and quantified by Labworks 4.0 software (UVP Inc.).

In vitro Kinase Assays

SW620 cells grown in 100-mm plates were treated with various concentration of epothilone B and D. Cell lysates for *in vitro* assays of IKKα and IKKβ kinase activities were prepared as described in Western blot analysis. *In vitro* kinase assays were done using immune complexes and bacterially synthesized glutathione *S*-transferase (GST)-IκBα or β proteins (2 μg) in 15 mL of kinase buffer containing 20 mmol/L HEPES (pH, 7.7), 2 mmol/L $MgCl_2$, 2 mmol/L MnCl, 10 μmol/L ATP, 5 μCi of [γ-^{32}P]ATP, 10 mmol/L β-glycerophosphate, 10 mmol/L NaF, 300 μmol/L Na_3VO_4, 1 mmol/L benzamidine, 2 μmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin, and 1 mmol/L DTT at 30°C for 30–60 min. Phosphorylated GST-IκBα and GST-IκBβ were separated by 12% SDS-PAGE, scraped into 96-well plates, and 100 μL cocktail was added to each well. IKKα and IKKβ kinase activities were quantified using a Liquid Scintillation Counter (Beckman-LS 6500, Beckman Instrument Inc.). Lysate samples were also separated on SDS/12%-polyacrylamide gel and then transferred to nitrocellulose. Membranes were probed with appropriate antibodies to detect IKKα and IKKβ as described for Western blot analysis above.

Transfection and Luciferase Activity Assays

The expression plasmids encoding IKKα (C178A)-Flag and IKKβ (C179A)-Flag were obtained from Dr. Dae-Myung Jue (Catholic University, Seoul, Korea; ref. 21). SW620 human colon cells (2.5×10^5 cells per well) were plated in 24-well plates and transiently transfected with IKK mutant plasmids using a mixture of plasmid and LipofectAMINE plus in OPTI-MEM according to the manufacturer's specifications (Invitrogen). Cell viabilities were determined, and confocal analysis of NF-κB (p50) translocation was done after treating transfected cells with epothilone B or D for designated times.

Tubulin Polymerization Assays

The polymerization of purified tubulin was monitored using CytoDYNAMIX Screen (Cytoskeleton). Tubulin polymerization was monitored spectrophotometrically at 340 nm. Absorbance was measured at 5-min intervals for 60 min using a Magellan microplate reader (TECAN Austria GmbH).

Immunofluorescent Labeling and Scanning-Laser Confocal Microscopy

SW620 cancer cells were cultured for 24 h on chamber slides (Lab-Tak II chamber slider system, Nunc Int.), fixed in 4% paraformaldehyde, membrane-permeabilized by exposure to 0.1% Triton X-100 in PBS for 30 min, and then placed in blocking serum (5% bovine serum albumin in PBS) at room temperature. Cells were then exposed to primary goat polyclonal antibody for p50 and p65 (1:100 dilution) or monoclonal anti–β-tubulin antibody (1:100) overnight at 4°C. After three washes with ice-cold PBS, cells were exposed to an anti-mouse IgG conjugated to Alexa® 488 (p65) or 594 (p50) anti-goat biotinylated secondary antibody (Molecular Probes) for 1 h at room temperature. Immunofluorescence images were acquired using a confocal laser-scanning microscope (TCS SP2, Leica Microsystems AG) at 630× magnification.

Terminal Nucleotidyl Transferase–Mediated Nick End Labeling Assay

To determine whether epothilone B and D induced cell death apoptosis, terminal nucleotidyl transferase–mediated nick end labeling (TUNEL) and 4′,6-diamidino-2-phenylindole (DAPI) staining were done. Briefly, SW620 human colon cancer cells were cultured on chamber slides. After treatment with epothilone B and D for 72 h, cells were washed twice with PBS and fixed in 4% paraformaldehyde in PBS for 1 h at room temperature. TUNEL assays were done using *in situ* cell death detection kits (Roche Diagnostics GmbH) according to the manufacturer's instructions. For DAPI staining, slides were incubated for 30 min at room temperature in the dark with mounting medium to measure DAPI fluorescence (Vector Laboratories Inc.). Cells were then observed under a fluorescence microscope (Zeiss). Total cell numbers in a given area was determined by DAPI nuclear staining. TUNEL-positive

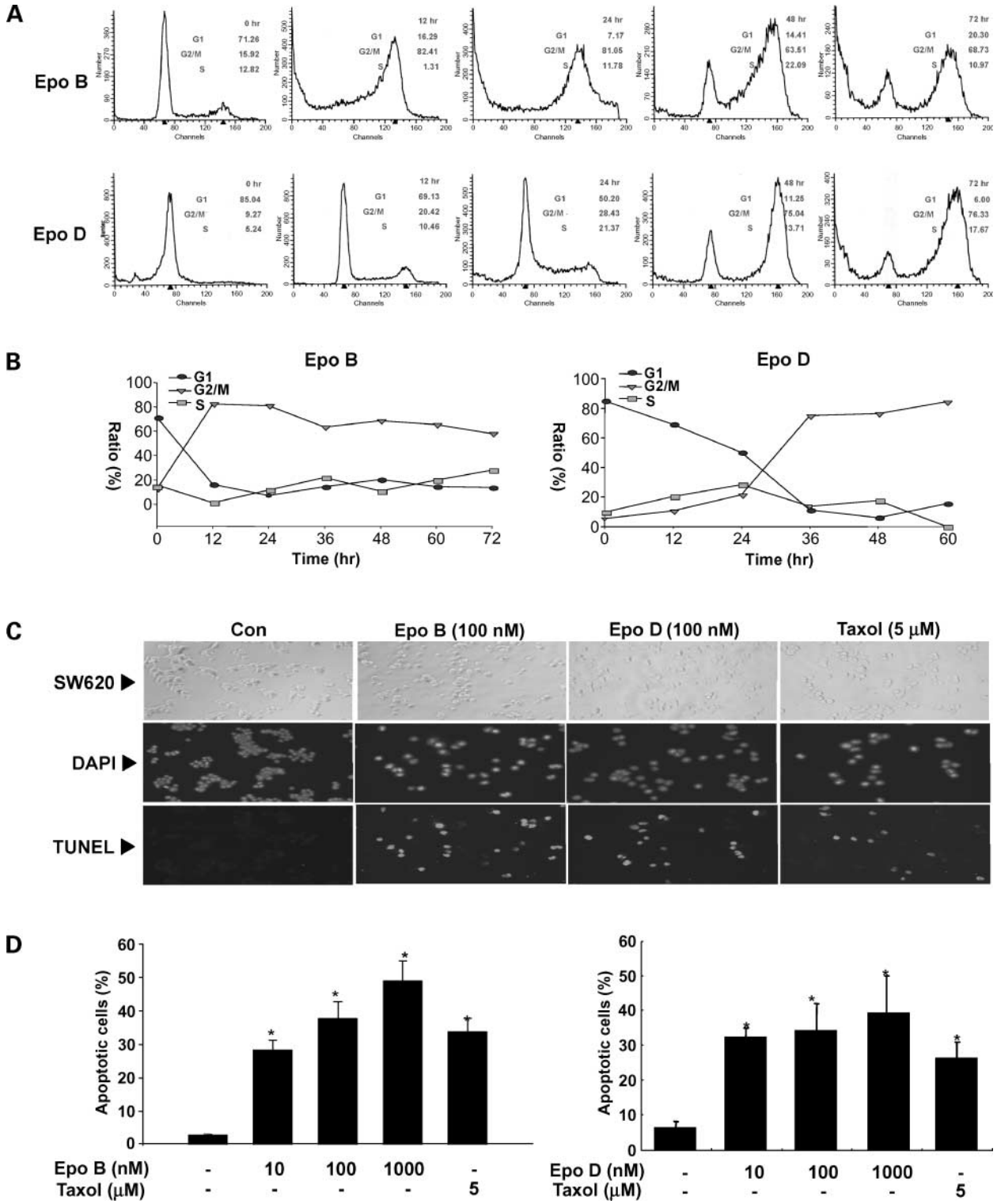

Figure 2. Cell cycle analysis, morphologic changes, and apoptotic cell death of SW620 cells treated with epothilones. SW620 cells were treated with 100 nmol/L of epothilone B or D for 72 h. DNA contents were analyzed by flow cytometry as described in Materials and Methods. Each panel is representative of three similar experiments done in triplicate. **A,** cell cycle arrest pattern analyzed by flow cytometry. **B,** percentage of cell distribution in the cell cycle. **C,** cell morphologic changes were observed under a microscope (**C,** *top,* magnification, 200×), and apoptotic cells were examined by fluorescence microscopy after TUNEL staining (**C,** *bottom*). Total cell numbers were determined by DAPI nuclear staining (**C,** *middle*). **D,** apoptotic index was defined as the number of TUNEL-positive stained cells divided by the number of DAPI-stained cells (fluorescence microscopy magnification, 100×). *Columns,* means of three experiments done in triplicate; *bars,* SD. *, $P < 0.05$, significantly different from the untreated group.

DAPI-stained cells were counted to quantify apoptosis. Percentages of apoptotic cells were expressed as ratios of TUNEL-positive cell numbers to DAPI-stained cell numbers.

Flow Cytometry Analysis

The effects of epothilone B and D on cell cycle distributions were determined by the flow cytometry of the nuclear DNA contents of cells after propidium iodide (PI) staining. Cells were treated with epothilone B and D (100 nmol/L) and incubated for different times (0–72 h). Cells were then harvested by trypsinization, washed twice with PBS, fixed in 1% formaldehyde, and dehydrated in 70% ethanol diluted with PBS. Cells were then incubated in PBS containing 100 μg/mL RNase and 40 μg/mL PI at 37°C for 1 h before flow cytometry. Cell distribution was determined using a Coulter Epics XL Flow Cytometer (Beckman Coulter).

Statistical Analysis

Data were analyzed by one-way ANOVA followed by Dunnet's test as a post hoc test. Differences were considered significant at the $P < 0.05$ level.

Results

Epothilone B and D Inhibited SW620 Colon Cancer Cell Growth

To determine the effects of epothilone B and D on cancer cell growth, SW620 human colon cancer cells were treated

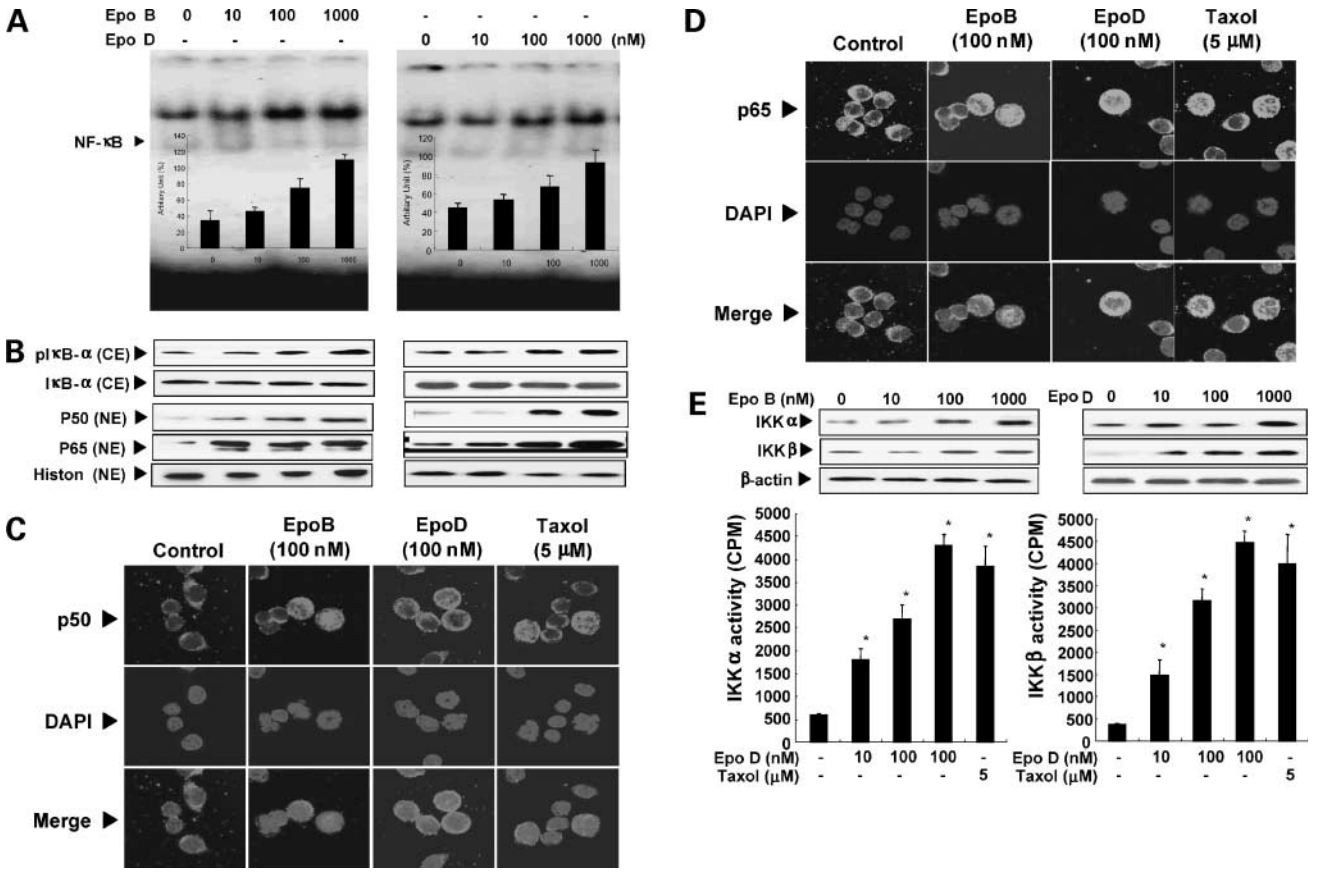

Figure 3. Effects of epothilone B or D on NF-κB activation in SW620 cells. **A,** the activation of NF-κB was determined by EMSA as described in Materials and Methods. Nuclear extracts of SW620 cells treated with epothilone B or D (10, 100, and 1000 nmol/L) for 1 h were incubated with ^{32}P–end-labeled oligonucleotide containing the κB sequence. NF-κB DNA binding activity was determined by EMSA. Densities of NF-κB DNA binding were calculated; *columns,* means of three independent experiments done in triplicate; *bars,* SD. *, $P < 0.05$, significantly different from the untreated group. **B,** effect of epothilone B or D on p50, p65, and phosphorylated IκB-α levels as assessed by Western blotting. Cells were treated with different concentrations of epothilone B and D (10, 100, and 1000 nmol/L) at 37°C for 1 h. Nuclear (for p50 and p65, *NE*), or cytosolic (for phosphorylated IκB, *CE*) proteins (50 μg) extracted after treatment were subjected to 12% SDS-PAGE. The expressions of p50, p65, IκB-α, β-actin, and histone H1 proteins were detected by Western blotting using specific antibodies. β-Actin and histone H1 proteins were used as internal controls. The panels shown are representative of three similar experiments done in triplicate. **C** and **D,** SW620 cells were treated with epothilone B or D at 37°C for 1 h. Cells were then washed and fixed, and the intracellular locations of p50 and p65 were determined by immunofluorescence using anti-p50 or -p65 antibodies followed by anti-mouse IgG secondary antibody conjugated with Alexa 488 for p65 or 594 for p50 as described in Materials and Methods. Pictures were taken using a confocal scanning microscope (original magnification, 630×). Double staining (Merge) with p50 or p65 and DAPI showed the localizations of p50 and p65 in the nucleus. **E,** SW620 cells were treated with epothilones for 1 h to determine IKK activity. IKKs were immunoprecipitated from treated cell lysates using anti-IKKα or anti-IKKβ antibodies and used for *in vitro* kinase reactions with GST-IκBα, GST-IκBβ, and [γ-^{32}P]ATP. GST-IκBα and GST-IκBβ phosphorylated by IKKs were determined by radioactivity counting (*CPM*) as IKKα and IKKβ activities (**E,** *bottom*) or visualized by SDS-PAGE followed by Western blotting (**E,** *top*). *Columns,* means of three independent experiments done in triplicate; *bars,* SD. *, $P < 0.05$, significantly different from the untreated group.

with increasing concentrations of epothilone B and D for up to 72 h, and cell survival was assessed by directly counting cells using the trypan blue dye exclusion method. As shown in Fig. 1A and B, treatment with epothilone B and D (0.1–1000 nmol/L) resulted in concentration- and time-dependent inhibitions in cell growth. Cell growth inhibitions by epothilone B and D were confirmed using WST-8 assays (data not shown).

Epothilone B and D Arrested SW620 Cells in G_2-M Phase

Given the potent inhibition of cell growth by epothilones, the effects of epothilone B or D on cell cycle distribution were examined to gain an insight into the inhibitory mechanism involved. As shown in Fig. 2, SW620 cells treated with epothilone B and D for 72 h were evaluated by PI staining–based fluorescence-activated cell sorting analysis to determine the effects of these agents. Epothilone B and D caused the rapid accumulation of cells in G_2-M at 12 h after treatment. Epothilone B caused early accumulation in the G_2-M phase at 12 h (80% of cell number were accumulated, Fig. 2A), whereas epothilone D caused accumulation at 36 h (when 75% of cells were involved, Fig. 2B). Accumulation of cells in G_2-M was accompanied by a reduction of cells in G_0-G_1. The percentage of cells in G_2-M was about 10–15% in untreated cells, but this gradually increased to about 70–80% after treatment with epothilones (Fig. 2B).

Epothilone B and D Induced Apoptotic Cell Death

To determine whether the inhibition of cell growth and cell arrest by these two epothilones were followed by the induction of apoptosis, we evaluated cell death by detecting changes in the chromatin morphology of cells using DAPI. To further characterize apoptosis by epothilone B or D, we did TUNEL staining assays and analyzed DAPI-positive TUNEL-labeled cells by fluorescence microscopy. Epothilone B and D–treated cells labeled by TUNEL showed increased fluorescence intensity (Fig. 2C). Apoptotic cells number (DAPI-positive TUNEL-stained cells) increased to 29%, 38%, and 49% after treatment with epothilone B at 10, 100, and 1,000 nmol/L, respectively (Fig. 2D). Percentages for epothilone D were 32%, 34%, and 39% at 10, 100, and 1000 nmol/L, respectively, whereas Taxol (5 μmol/L) induced about 25–32% apoptotic cell death (Fig. 2D).

Epothilone B or D Induced NF-κB Activation

NF-κB is known to regulate the apoptosis of several cancer cell lines, and tubulin-disrupting anticancer drugs, such as nocodazole, vincristine, vinblastine, and paclitaxel, activate intracellular signaling pathways involved in the activation of transcription factor NF-κB. To determine whether the activation of NF-κB is also involved in epothilone-induced apoptotic cell death, we determined the DNA binding activities of NF-κB using electrophoretic gel mobility shift assays. It was found that NF-κB had DNA binding activity in untreated SW620 cells, and that treatment with epothilone B or D increased this DNA binding activity in a dose-dependent manner (Fig. 3A). Tumor necrosis factor-α (TNF-α) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treatment as positive controls also increased NF-κB activity (data not shown). To further investigate the mechanism of NF-κB activation by these epothilones, we analyzed the effects of epothilone B and D on the nuclear translocations of p50 and p65 and on IκBα protein degradation by Western blotting and confocal microscopy. As shown in Fig. 3B, epothilone B and D promoted nuclear translocations of p50 and p65 into the nucleus. The effects of epothilone B or D on the translocations of p50 and p65 were also confirmed by confocal microscopy. Significant increased merged fluorescence image of p65 (Green, Alexa Fluor 594 dye stained) or p50 (Red, Alexa Fluor 488 dye stained) with nucleus (DAPI staining, Blue) was observed (Fig. 3C and D). Concomitant with the nuclear translocations of p50 and p65, levels of phosphorylated IκB were found to be increased in the cytosol. These results indicate that epothilone B and D both promote the nuclear translocations of p50 and p65 by inducing IκB phosphorylation; paclitaxel (Taxol, 5 μmol/L) was found to have a similar effect. Moreover, TNF-α–induced NF-κB was also significantly inhibited by epothilone B and D (data not shown).

The IKK complex (IKKα/IKKβ) is responsible for the phosphorylation and degradation of IκB, which is required for the activation of NF-κB. To determine whether epothilone-induced NF-κB activity is due to IκB up-regulation via IKK activation, we determined IKK activities in cells treated with epothilones using the same conditions used for NF-κB activation. Epothilone B and D increased IKKα and β activities dose dependently (Fig. 3E), which was consistent NF-κB activation and IκB release. To confirm that epothilone-increased IKK activities are required for epothilone-induced apoptosis, cell growth inhibitions were evaluated in the presence of sodium salicylic acid (an IKK inhibitor). Treatment with sodium salicylic acid ameliorated epothilone B–induced cell growth inhibition dose dependently and also reduced epothilone D–induced cell growth, although not dose dependently (Fig. 4A and B). It has been reported that the cysteine residues of IKKs are important for activity, and that many agents that inhibit the activity of IKKs modify their cysteine residues (17, 21). Thus, we expressed wild-type and mutant forms of IKKα (C178A) and IKKβ (C179A), in which cysteine residues were replaced with alanine, in SW620 cells, and then determined the effects of epothilone B or D on p50 translocation to the nucleus and on cell viability. As shown in Fig. 4C, epothilone B did not inhibit cell growth in these IKKα and IKKβ mutant transfected cells. In addition, we did not observe merged fluorescence images of p50, suggesting that epothilone B or D did not translocate p50 in IKKα (Fig. 4D) or IKKβ (data not shown) mutant transfected cells. These results suggest that the activation of IKKs plays a critical role in the epothilone-induced apoptosis of SW620 cells.

Effect of Epothilones on Cellular Microtubule Polymerization

Because epothilone treatment markedly blocked the cell cycle at G_2-M, we examined whether the epothilone-induced disruption of tubulin polymerization could directly affect

SW620 apoptosis. To test this hypothesis, we studied tubulin polymerization *in vitro* at room temperature in a reaction mixture containing purified tubulin and GTP in the presence or absence of epothilone B. The results, presented in Fig. 5A, show that epothilone B significantly promoted tubulin polymerization in a concentration-dependent manner. Moreover, because strong paclitaxel-like tubulin-stabilizing activity was found *in vitro*, we investigated whether epothilone treatment affected the cellular microtubule network. Cells were treated with 100 nmol/L of epothilone B and D for 24 h, and then microtubule networks were visualized immunocytochemically. Microtubule networks in control cells exhibited normal arrangements and organization, whereas treatment with epothilones resulted in microtubule polymerization with an increase in the density of cellular microtubules and the formation of long thick microtubule bundles surrounding the nucleus (Fig. 5B), and paclitaxel produced similar effects. It has been reported that tubulin polymerization can cause apoptosis by causing NF-κB translocation to the nucleus, where tubulin and NF-κB form a complex known to regulate apoptosis. To study this possibility, tubulin polymerization was tested in the presence of sodium salicylic acid. However, this IKK inhibitor did not change epothilone B–induced tubulin polymerization. We also examined whether epothilone-induced tubulin polymerization and NF-κB (p50) translocation co-occurred in the nucleus. To this end, we examined the colocalizations of p50 and tubulin. In SW620 cells treated with epothilone B for 24 h, tubulin polymerization was evident, and translocated p50 was observed in the nucleus (Fig. 5B). Consistent with the observed cell growth inhibition, p50 and polymerized tubulin colocalization was present in the nucleus after epothilone B and D treatments. However, in the presence of sodium salicylic acid, epothilone-induced p50 was absent, although tubulin polymerization was observed (Fig. 5Ca). In addition, epothilone B– or D–induced p50 translocation was inhibited in mutant IKK

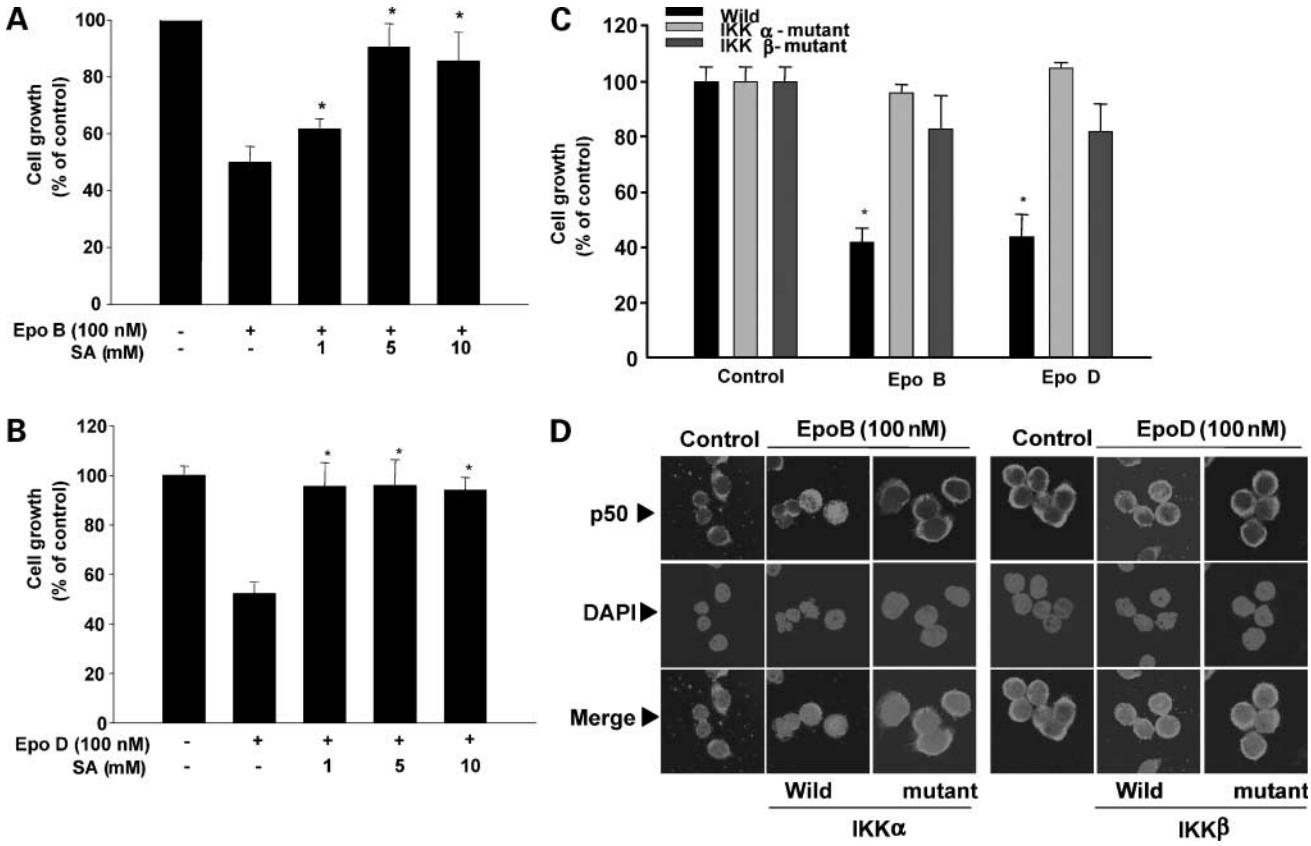

Figure 4. Effect of IKK inhibitor (sodium salicylic acid, *SA*) and mutant IKKs on epothilone-induced cell growth inhibition and NF-κB (p50) translocation. **A** and **B,** SW620 human colon cancer cells were treated with epothilone B or D (100 nmol/L) for 72 h and assayed for cell growth. Cells were then pretreated with salicylic acid (1, 5, 10 mmol/L) for 1 h before epothilone treatments. *Columns,* means of three experiments done in triplicate; *bars,* SD. *, $P < 0.05$, significantly different from the epothilone (*Epo*)-treated group. **C,** SW620 cells were transiently transfected with wild or mutant IKKs for 24 h and then treated with epothilone B or D for 72 h to determine cell growth inhibitions. *Columns,* means of three independent experiments done in triplicate; *bars,* SD. *, $P < 0.05$, significantly different from the wild-type untreated control group. **D,** SW620 cells were transiently transfected with wild or mutant IKKs for 24 h and then treated with epothilone B or D for 1 h to follow p50 translocation. Cells were washed and fixed, and the intracellular locations of p50 were determined by immunofluorescence using an anti-p50 antibody followed by an anti-mouse IgG secondary antibody conjugated with Alexa 594 as described in Materials and Methods. Pictures were taken using a confocal scanning microscope (original magnification, 630×). Double staining (*Merge*) for p50 and DAPI showed the nuclear localization of p50. Panels are representative of at least three experiments.

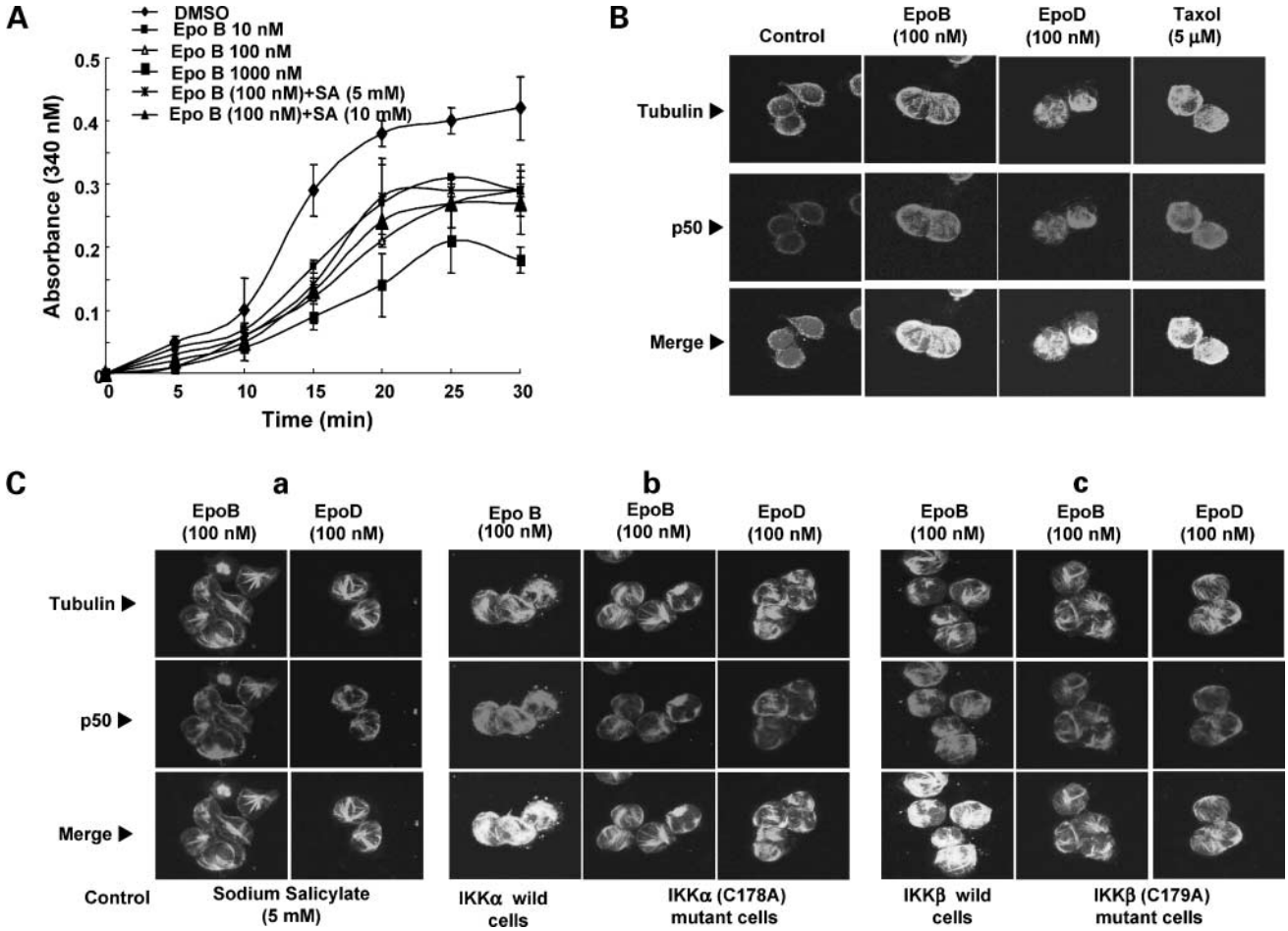

Figure 5. Effect of epothilone B or D on tubulin polymerization and the colocalization of β-tubulin and p50. **A,** tubulin in reaction buffer was incubated at 37°C in the presence of vehicle (*DMSO*) or epothilone B and D, and microtubule assembly was then measured spectrophotometrically as described in Materials and Methods. Values are of three independent experiments done in triplicate. **B,** to determine the colocalization of tubulin and p50, cells treated with epothilone B or D for 24 h were fixed in 4% paraformaldehyde, membrane-permeabilized with 0.2% Triton X-100 for 5 min in PBS, and placed in blocking serum containing 5% horse or goat serum in PBS. Cells were then stained with monoclonal anti-tubulin/Alexa 488–conjugated anti-mouse IgG and with monoclonal antibody against p50 followed by anti-mouse IgG Alexa 594. Immunofluorescence images were acquired using a confocal laser scanning microscope (dual wavelength scan) with a 360× oil immersion objective. The confocal laser scanning micrograph shows a merged image double-labeled with tubulin and p50 antibodies. Triple staining (*Merge*) for tubulin or p50 and DAPI demonstrating the nuclear colocalization of tubulin-p50 complex. Panels are representative of three different experiments. **Ca,** effect of sodium salicylic acid on the epothilone-induced colocalization of tubulin and p50. SW620 cells were pretreated with salicylic acid (5 mmol/L) for 1 h before adding epothilones and then further cultured for 24 h with epothilone B or D (100 nmol/L). **Cb** and **Cc,** SW620 cells were transiently transfected with wild or mutant types of IKKα (**Cb**) and β (**Cc**) for 24 h, and then cells were treated with epothilone B or D for 24 h to determine the colocalization of tubulin and p50. Panels are representative of three different experiments.

(C178A or C179A) transfected SW620 cells, whereas tubulin polymerization was not inhibited (Fig. 5Cb and c). However, epothilone increased tubulin polymerization and p50 translocation into the nucleus in the cells transfected wild-type IKKs, which showed the combined yellow color images, meaning the two proteins are merged. These results suggest that epothilone-induced cell growth inhibition (or apoptotic cell death) is not related to tubulin polymerization.

Epothilone B and D Induced the Expression of Apoptotic Cell Death Regulatory Proteins

It has been shown that NF-κB plays an important role in coordinating the control of apoptotic cell death (11, 12). Several apoptosis-associated genes or regulatory proteins, such as, p53, Bax, Bcl-2, and caspase-3, have been reported to be activated or down-regulated by NF-κB in various tumor cells. To determine whether any of these proteins are regulated by NF-κB induced by epothilones and whether they are involved in the mediation of epothilone-induced apoptosis, we evaluated their gene expressions in SW620 cells treated with the epothilone B or D and then examined whether changes in their protein expressions were altered by sodium salicylic acid. The expressions of the proapoptotic proteins, Bax, p53, and the active form of caspase-3 were increased, but the expression of antiapoptotic Bcl-2 was decreased after epothilone B or D

treatment in a dose-dependent manner (Fig. 6A). Moreover, these increased protein expressions were decreased in cells cotreated with sodium salicylic acid, whereas Bcl-2 expression was increased (Fig. 6B). In addition, it was also found that sodium salicylic acid reduced epothilone-induced NF-κB activity (Fig. 6C). These results suggest that the expression of apoptosis-related proteins by epothilones is regulated via IKK-dependent NF-κB activation. To further investigate which cell death regulatory protein is predominantly involved in epothilone-induced apoptosis and NF-κB activation, we cotreated with SW620 cells with epothilone B (100 nmol/L) and p53 inhibitor or caspase-3 inhibitor or Bax inhibitor for 72 h to assess cell death or for 1 h to assess NF-κB activity. It was found that caspase-3 inhibitor prevented most the epothilone-induced cell death (Fig. 6Da) and NF-κB activity (Fig. 6Db), suggesting that NF-κB/IKK–dependent caspase-3 pathway is most involved in epothilone-induced apoptosis.

Discussion

In this study, it was found that epothilone B or D induced cell growth arrest followed by apoptotic cell death in SW620 colon cancer cells, and that this apoptotic cell death is associated with NF-κB activation via the release of IκB through the activation of IKKs. These epothilones also induced several NF-κB–dependent apoptotic regulatory

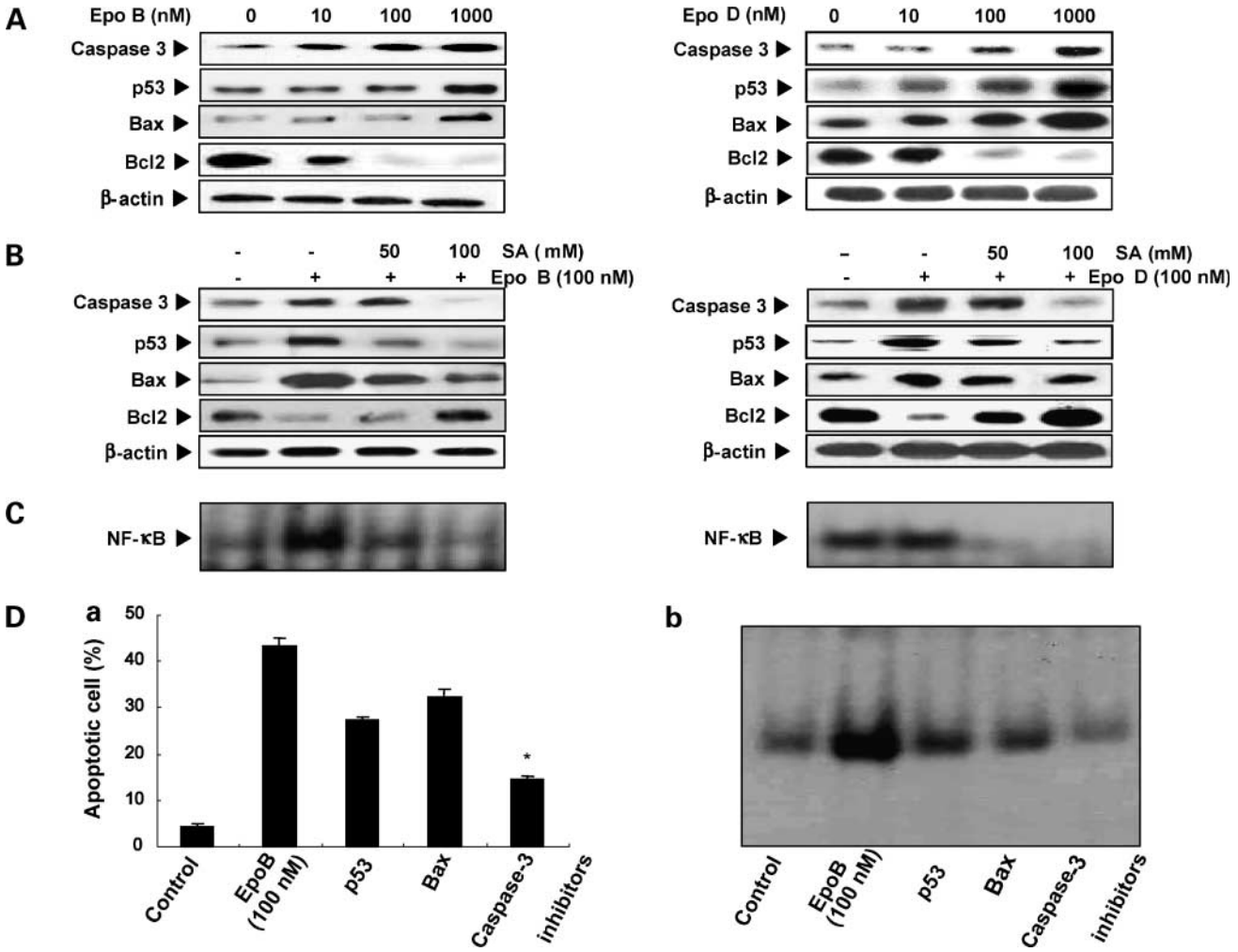

Figure 6. Effects of epothilone B or D, IKK inhibitor, and cell death regulatory protein inhibitor on the expressions of apoptosis regulatory proteins (**A** and **B**), NF-κB activation (**C** and **Db**), and on cell death (**Da**). **A,** cells were treated with different concentrations (0, 10, 100, and 1000 nmol/L) of epothilone B and D at 37°C for 24 h. Equal amounts of total proteins (50 μg per lane) were subjected to 12% SDS-PAGE. The expressions of Bcl-2, Bax, p53, caspase-3, caspase-9, and β-actin were detected by Western blotting using specific antibodies. β-Actin protein was used as an internal control. **B** and **C,** effect of IKK inhibitor (sodium salicylic acid) on the expressions of apoptosis-regulating proteins (**B**) and on NF-κB activation (**C**). SW620 cells were pretreated with sodium salicylic acid (1, 5, 10 mmol/L) for 1 h before adding epothilone B or D (100 nmol/L). **D,** effect of cell death regulatory protein inhibitors on epothilone B–induced cell death (**Da**) and on the activation of NF-κB (**Db**). SW620 cells were pretreated with several inhibitors [50 μmol/L z-DEVD-FMK (a specific caspase-3 inhibitor), 50 μmol/L pifithrin-α (a specific p53 inhibitor), or 50 μmol/L peptide P5 (Pro-Met-Leu-Lys-Glu; a specific Bax inhibitor)] of cell death regulatory proteins 1 h before epothilone B or D treatment (100 nmol/L). Cell death was determined 72 h later. NF-κB activation was determined 1 h after adding the epothilones. Panels are representative of three similar experiments done in triplicate.

genes, i.e., p53, caspase-3, and Bax, but attenuated Bcl-2 expression. In addition, the expressions of these apoptotic cell death regulatory genes were prevented in cells treated with sodium salicylic acid (an IKK inhibitor) and in cells expressing mutant IKKs without affecting epothilone-induced tubulin polymerization. Moreover, the caspase-3 inhibitor most effectively reversed epothilone B–induced cell death and NF-κB activity. These results show that epothilone-induced SW620 colon cancer cell apoptosis may be due to the activation of NF-κB/IKK–dependent caspase-3 apoptotic cell death signals without affecting tubulin polymerization.

Epothilone B or D caused an accumulation of SW620 colon cancer cells in the G_2-M phase. Epothilone B rapidly caused cell arrest after 12 h of treatment, whereas epothilone D caused an accumulation of cells in the G_2-M phase at 36 h. Although times required to cause cell arrest differed for epothilone B and D, cell growth arrest continued after both for 72 h, and this was followed by apoptotic induction at 72 h.

The tubulin polymerization is required for division, but increase of the tubulin polymerization (hyper-polymerization) prevents cell division that could cause cell arrest followed by cell death. It is the main rationality that anticancer drugs such as paclitaxel and docetaxel and other tubulin stabilizers could prevent cell growth via the interference of cell division in the rapidly replicated cells (cancer cells). It is possible that cell growth inhibition by epothilone resulted in the induction of apoptosis because epothilone significantly arrested cell cycle in G_2-M phase, which could be followed by the induction of apoptosis. The data also further confirmed that the cell growth inhibition by epothilones is about 60–70%, which is almost according to the similar portion with apoptotic cell death by epothilones.

Evidence now available indicates that the activation of NF-κB plays an important role in coordinating apoptotic cell death (11, 12). However, it has been reported that NF-κB activation can promote or prevent apoptosis, and that this depends on the stimulus and cell type (22–25). Moreover, although it has been reported that rearrangement of the β-tubulin cytoskeleton by microtubule-disrupting agents is necessary for the nuclear translocation of NF-κB (10), it is unclear whether NF-κB activation is critical for microtubulin-disrupting agents–induced cell death. To determine whether NF-κB activation is associated with epothilone-induced apoptotic cell death, we first examined the DNA binding activity of NF-κB and its translocation. As was expected, both were significantly increased in parallel with an increase in phosphorylated IκB in cytosol after treatment with both epothilones. Recently, several studies have shown that IKKs (IKKα and IKKβ) play a key role in the phosphorylation and degradation of IκBs (26, 27). Therefore, we examined the effects of epothilones on IKK activity. *In vitro* IκB kinase assays showed that IKK were significantly activated by epothilones, and in another study, treatment with sodium salicylic acid (an IKK inhibitor) prevented epothilone-induced IκBα release, NF-κB (p50) translocation, and apoptosis. By transfecting mutant IκBα and β containing a vector in which cysteine residues of IKKα (C179A) and IKKβ (C178A) were replaced with alanine into SW620 colon cancer cells, we also found that transfectants were insensitive to epothilone-induced IKK-mediated NF-κB (p50) translocation and apoptosis. Moreover, substitution of these residues with other amino acids has been reported to prevent the signal-induced phosphorylation of IκBα, thereby inhibiting NF-κB activation (17, 21). These findings further indicate that epothilone-stimulated IKK activation is critical for NF-κB (p50) translocation and the consequent regulation of apoptotic cell death.

How epothilone B and D react with IKKs remains unclear, but it is noteworthy that a number of other reactive compounds, e.g., the sesquiterpene lactones (parthenolide, helenalin, and 4β,15-epoxy-miller-9*E*-enolide; refs. 26–29), cyclopentenone prostaglandins (prostaglandin A_1 and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, kamebakaurin), arsenite, and the gold-containing compound auranofin (17, 21, 30–32), react with cysteine to inhibit NF-κB/IKK activity. Natural products containing epoxides or α,β-unsaturated ketones are known to react with nucleophilic functionalities like thiols that are present in biomolecules (20, 33–35). However, it is not clear how epothilones modify single protein targets; thus, the identification of the complete range of epothilone molecular targets would probably shed light on its molecular mechanism(s). In agreement with our findings, several recent studies have suggested that NF-κB activation is critical for the cancer cell death caused by tubulin polymerization agents (14–16, 18). Huang and Fan reported that tumor cells transfected with antisense IκBα showed enhanced sensitivity to paclitaxel-induced apoptotic cell death, which suggests that paclitaxel-activated NF-κB is important for the apoptosis of solid tumor cells by paclitaxel (14). Huang et al. (18) also found that *Vinca* alkaloid induced human tumor cell death through the NF-κB pathway activation. These results suggest that NF-κB and IKKs activation may be important for epothilone-induced colon cancer cell death, and that the proteolytic degradation of IκBα via IKK activation might be an important step in the activation of NF-κB.

Disruption of the dynamic reorganization of the microtubule network microtubulin-disrupting agents results in aberrant mitotic formation and prevents cells transversing from metaphase to anaphase, and ultimately, the prolonged mitotic arrest of many tumor cell types leads to apoptosis. These results thus suggest that tubulin polymerization may be a prerequisite of epothilone-induced apoptotic cell death. In this study, although epothilone B and D both induced tubulin polymerization, cells treated with sodium salicylic acid or transfected with mutant IKKs were unaffected by epothilone-induced NF-κB activity and did not undergo apoptosis; however, tubulin polymerization was unchanged, which suggests that epothilone-induced apoptotic cell death may occur via a mitotic arrest–independent NF-κB pathway. Mitotic arrest–independent apoptotic pathways induced by anti-microtubule agents

like paclitaxel, *Vinca* alkaloids, docetaxel, and nocodazole have been previously described (14–16, 18). However, based on these observations, the identities of the possible primary molecular targets of epothilones that mediate the activation of the NF-κB signaling cascade remain unclear if the epothilone-induced activation of NF-κB is independent of microtubule polymerization. Several studies have shown that cell cycle arrest followed by apoptotic cell death as induced by anti-microtubule drugs is associated with a number of signaling pathways, which include the phosphorylation of Bcl-2 and Cdc25C, the expression of survivin and cell death regulatory genes (e.g., Bax and p53) and of caspase cascades, and activation of several kinase pathways (protein kinase A and C and c-Jun NH_2-terminal kinase; refs. 36–38). Recently, it has been revealed that many these potential target genes can be induced by NF-κB activation during the apoptosis of cancer cells (22, 26, 39). In the present study, we also found that epothilones up-regulated the expressions of caspase-3, p53, and Bax, but down-regulated Bcl-2, and sodium salicylic acid was found to interfere with these effects of epothilones on p53, caspase, Bax, and Bcl-2 in parallel with inactivating NF-κB. These findings suggest that epothilones might induce apoptotic cell death by activating NF-κB and IKK-mediated apoptotic signals. Therefore, it is possible that NF-κB/IKK is a primary target during apoptotic gene induction by epothilone. We also showed that the caspase-3–specific inhibitor most effectively reversed epothilone B–induced apoptotic cell death and NF-κB activation in SW620 cells, which suggests that the activation of NF-κB/IKK–dependent caspase-3 could represent a significant signal during epothilone-induced apoptosis. In agreement with this finding, the significance of caspase activity during microtubulin-disrupting agent–induced apoptosis has been reported during the nocodazole-induced apoptosis of lymphocytic leukemia cells (40).

Summarizing, epothilone treatment seems to lead to IKK activity enhancement, which causes IκB degradation. Released cytoplasmic NF-κB (p50) then translocates to the nucleus, where it functions as an important transcription factor and regulates cell death genes. These results suggest that epothilones induce apoptosis via the activation of NF-κB/IKK signaling pathway in a tubulin polymerization–independent manner.

References

1. Jordan MA, Wilson L. Microtubules and actin filaments: dynamic targets for cancer chemotherapy. Curr Opin Cell Biol 1998;10:123–30.

2. Watkins EB, Chittiboyina AG, Jung JC, Avery MA. The epothilones and related analogues—a review of their syntheses and anti-cancer activities. Curr Pharm Des 2005;11:1615–53.

3. Wu KD, Cho YS, Katz J, et al. Investigation of antitumor effects of synthetic epothilone analogs in human myeloma models *in vitro* and *in vivo*. Proc Natl Acad Sci U S A 2005;102:10640–5.

4. Goodin S, Kane MP, Rubin EH. Epothilones: mechanism of action and biologic activity. J Clin Oncol 2004;22:2015–25.

5. Earhart RH. Docetaxel (Taxotere): preclinical and general clinical information. Semin Oncol 1999;26:8–13.

6. Mann J. Natural products in cancer chemotherapy: past, present and future. Nat Rev Cancer 2002;2:143–8.

7. Wang LG, Liu XM, Kreis W, Budman DR. The effect of antimicrotubule agents on signal transduction pathways of apoptosis: a review. Cancer Chemother Pharmacol 1999;44:355–61.

8. Bhalla KN. Microtubule-targeted anticancer agents and apoptosis. Oncogene 2003;22:9075–86.

9. Bourgarel-Rey V, Vallee S, Rimet O, et al. Involvement of nuclear factor κB in c-Myc induction by tubulin polymerization inhibitors. Mol Pharmacol 2001;59:1165–70.

10. Bava SV, Puliappadamba VT, Deepti A, Nair A, Karunagaran D, Anto RJ. Sensitization of taxol-induced apoptosis by curcumin involves down-regulation of nuclear factor-κB and the serine/threonine kinase Akt and is independent of tubulin polymerization. J Biol Chem 2005;280:6301–8.

11. Viatour P, Merville MP, Bours V, Chariot A. Phosphorylation of NF-κB and IκB proteins: implications in cancer and inflammation. Trends Biochem Sci 2005;30:43–52.

12. Baldwin AS. The NF-κB and IκB proteins: new discoveries and insights. Annu Rev Immunol 1996;14:649–83.

13. Huang Y, Johnson KR, Norris JS, Fan W. Nuclear factor-κB/IκB signaling pathway may contribute to the mediation of paclitaxel-induced apoptosis in solid tumor cells. Cancer Res 2000;60:4426–32.

14. Huang Y, Fang Y, Dziadyk JM, Norris JS, Fan W. The possible correlation between activation of NF-κB/IκB pathway and the susceptibility of tumor cells to paclitaxel-induced apoptosis. Oncol Res 2002;13:113–22.

15. Huang Y, Fan W. IκB kinase activation is involved in regulation of paclitaxel-induced apoptosis in human tumor cell lines. Mol Pharmacol 2002;61:105–13.

16. Fan W, Sui M, Huang Y. Glucocorticoids selectively inhibit paclitaxel-induced apoptosis: mechanisms and its clinical impact. Curr Med Chem 2004;11:403–11.

17. Kapahi P, Takahashi T, Natoli G, et al. Inhibition of NF-κ B activation by arsenite through reaction with a critical cysteine in the activation loop of IκB kinase. J Biol Chem 2000;275:36062–6.

18. Huang Y, Fang Y, Wu J, et al. Regulation of *Vinca* alkaloid–induced apoptosis by NF-κB/IκB pathway in human tumor cells. Mol Cancer Ther 2004;3:271–7.

19. Lee HY, Hong YD, Lee-Choi IY. Synthesis toward epothilone A: coupling reaction between the sulfone of C1–10 and the allylic bromide of C11–21. Bull Korean Chem Soc 1999;20:403–4.

20. Lee SH, Lee CW, Lee JW, et al. Induction of apoptotic cell death by 2′-hydroxycinnamaldehyde is involved with ERK-dependent inactivation of NF-κB in TNF-α-treated SW620 colon cancer cells. Biochem Pharmacol 2005;70:1147–57.

21. Jeon KI, Byun MS, Jue DM Gold compound auranofin inhibits IκB kinase (IKK) by modifying Cys-179 of IKKβ subunit. Exp Mol Med 2003;35:61–6.

22. Bellarosa D, Binaschi M, Maggi CA, Goso C. Sabarubicin (MEN 10755) and paclitaxel show different kinetics in nuclear factor-κB (NF-κB) activation: effect of parthenolide on their cytotoxicity. Anticancer Res 2005;25:2119–28.

23. Chen GG, Liang NC, Lee JF, et al. Over-expression of Bcl-2 against *Pteris semipinnata* L.–induced apoptosis of human colon cancer cells via a NF-κB–related pathway. Apoptosis 2004;9:619–27.

24. Rouet-Benzineb P, Aparicio T, Guilmeau S, et al. Leptin counteracts sodium butyrate-induced apoptosis in human colon cancer HT-29 cells via NF-κB signaling. J Biol Chem 2004;279:16495–502.

25. Nakahara C, Nakamura K, Yamanaka N, et al. Cyclosporin-A enhances docetaxel-induced apoptosis through inhibition of nuclear factor-κB activation in human gastric carcinoma cells. Clin Cancer Res 2003;9:5409–16.

26. Farhana L, Dawson MI, Fontana JA. Apoptosis induction by a novel retinoid-related molecule requires nuclear factor-κB activation. Cancer Res 2005;65:4909–17.

27. Hu MC, Hung MC. Role of IκB kinase in tumorigenesis. Future Oncol 2005;1:67–78.

28. Hehner SP, Hofmann TG, Droge W, Schmitz ML. The antiinflammatory sesquiterpene lactone parthenolide inhibits NF-κB by targeting the IκB kinase complex. J Immunol 1999;163:5617–23.

29. Koch E, Klaas CA, Rungeler P, et al. Inhibition of inflammatory cytokine production and lymphocyte proliferation by structurally different

sesquiterpene lactones correlates with their effect on activation of NF-κB. Biochem Pharmacol 2001;62:795 – 801.

30. Lee JH, Koo TH, Hwang BY, Lee JJ. Kaurane diterpene, kamebakaurin, inhibits NF-κB by directly targeting the DNA-binding activity of p50 and blocks the expression of antiapoptotic NF-κB target genes. J Biol Chem 2002;277:18411 – 20.

31. Cernuda-Morollon E, Pineda-Molina E, Canada FJ, Perez-Sala D. 15-Deoxy-Δ12,14-prostaglandin J2 inhibition of NF-κB-DNA binding through covalent modification of the p50 subunit. J Biol Chem 2001;276:35530 – 6.

32. Liang MC, Bardhan S, Pace EA, et al. Inhibition of transcription factor NF-κB signaling proteins IKKβ and p65 through specific cysteine residues by epoxyquinone A monomer: correlation with its anti-cancer cell growth activity. Biochem Pharmacol 2006;71:634 – 45.

33. Liang MC, Bardhan S, Li C, Pace EA, Porco JA, Jr., Gilmore TD. Jesterone dimer, a synthetic derivative of the fungal metabolite jesterone, blocks activation of transcription factor nuclear factor κB by inhibiting the inhibitor of κB kinase. Mol Pharmacol 2003;64:123 – 31.

34. Alfonso J, Garcia P, Victor C, Gerardo M, Thomas JS, Elisabeth S. Cysteine 38 in p65/NF-κB plays a crucial role in DNA binding inhibition by sesquiterpene lactones. J Biol Chem 2001;276:39713 – 20.

35. Ji C, Kozak KR, Marnett LJ. IκB kinase, a molecular target for inhibition by 4-hydroxy-2-nonenal. J Biol Chem 2001;276:18223 – 8.

36. Huang YT, Huang DM, Guh JH, Chen IL, Tzeng CC, Teng CM. CIL-102 interacts with microtubule polymerization and causes mitotic arrest following apoptosis in the human prostate cancer PC-3 cell line. J Biol Chem 2005;280:2771 – 9.

37. Jackson SJ, Singletary KW. Sulforaphane: a naturally occurring mammary carcinoma mitotic inhibitor, which disrupts tubulin polymerization. Carcinogenesis 2004;25:219 – 27.

38. Ling YH, Jiang JD, Holland JF, Perez-Soler R. Arsenic trioxide produces polymerization of microtubules and mitotic arrest before apoptosis in human tumor cell lines. Mol Pharmacol 2002;62:529 – 38.

39. Ho WC, Dickson KM, Barker PA. Nuclear factor-κB induced by doxorubicin is deficient in phosphorylation and acetylation and represses nuclear factor-κB – dependent transcription in cancer cells. Cancer Res 2005;65:4273 – 81.

40. Beswick RW, Ambrose HE, Wagner SD. Nocodazole, a microtubule de-polymerising agent, induces apoptosis of chronic lymphocytic leukaemia cells associated with changes in Bcl-2 phosphorylation and expression. Leuk Res 2006;30:427 – 36.

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