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NF-κB Pathway Is Involved in Griseofulvin-Induced G2/M Arrest and Apoptosis in HL-60 Cells

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Abstract Griseofulvin (GF), an oral antifungal agent, has been shown to exert antitumorigenesis effect through G2/M cell cycle arrest in colon cancer cells. But the underlying mechanisms remained obscure. The purpose of this study is to test the cytotoxic effect of GF on HL-60 and HT-29 cells and elucidate its underlying molecular pathways. Dose-dependent and time-course studies by flow cytometry demonstrated that 30 to 60 μM GF significantly induced G2/M arrest and to a less extend, apoptosis, in HL-60 cells. In contrast, only G2/M arrest was observed in HT-29 cells under similar condition. Pretreatment of 30 μM TPCK, a serine protease inhibitor, completely reversed GF-induced G2/M cell cycle arrest and apoptosis in HL-60 cells but not in HT-29 cells. The GF-induced G2/M arrest in HL-60 cells is reversible. Using EMSA and super-shift analysis, we demonstrated that GF stimulated NF-κB binding activity in HL-60 cells, which was completely inhibited by pretreatment of TPCK. Treatment of HL-60 with 30 μM GF activated JNK but not ERK or p38 MAPK and subsequently resulted in phosporylation of Bcl-2. Pretreatment of TPCK to HL-60 cells blocked the GF-induced Bcl-2 phosphorylation but not JNK activation. Time course study demonstrated that activation of cdc-2 kinase activity by GF correlated with Bcl-2 phosphorylation. Taken together, our results suggest that activation of NF-κB pathway with cdc-2 activation and phosphorylation of Bcl-2 might be involved in G2/M cell cycle arrest in HL-60 cells. J. Cell. Biochem. 101: 1165–1175, 2007.

Key words: griseofulvin; G2/M arrest; NF-κB; HL-60

Griseofulvin (GF), an antifungal antibiotic produced by various species of *Penicillium*, has been extensively used in the therapy of dermatophytoses by oral administration [De Carli and Larizza, 1988]. GF is metabolized in liver microsome through NADPH-mediated dealkylation and excreted as 6-demethylgriseofulvin in the urine [Symchowicz and Wong, 1966]. The biological activity of GF is manifested as a spindle poison that interferes with the organization of microtubules at mitosis or other processes mediated by the microtubule network [Malawista et al., 1968; Grisham et al., 1973; Weber et al., 1976; Wehland et al., 1977]. It has been demonstrated that microtubule-active drugs continue to be one of the most successful cancer chemotherapeutic agents [Jordan and Wilson, 2004]. Our report also demonstrated that GF-induced G2/M cell cycle arrest and abnormal microtubule polymerization in cancer cells and potentiated nocodazole-induced anticancer effect suggesting the potential application of GF [Ho et al., 2001]. But the signal pathways involved in the GF-induced G2/M cell cycle arrest are still not fully understood.

There are multiple checkpoint systems to control mitosis [Clarke and Gimenez-Abian,

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2000]. The mitotic kinase, cylin B1-cdc2, is thought to be involved in the regulation of G2/M arrest which is important for preventing mitotic entry when DNA is damaged [Matsuoka et al., 1998]. This process is accomplished by activation of checkpoint kinase 1 or 2 (Chk1 or Chk2) to phosphorylate cdc25C on serine 216 and prevents cdc25C from activation of cylin B1-cdc2 [Walworth and Bernards, 1996; Furnari et al., 1997; Sanchez et al., 1997]. Microtubule-interfering agents including paclitaxel, vinca alkaloids, colchicine, and GF-induced cell cycle arrest at G2/M phase and initiation of apoptosis [Bonfoco et al., 1995; Tishler et al., 1995; Huschtscha et al., 1996; Ho et al., 2001]. Previous report demonstrated that JNK/SAPK pathway was involved in the regulation of cell cycle [Shim et al., 1996] and that microtubule-interfering agents activates JNK/SAPK in a variety of human cells [Wang et al., 1998] suggesting the possible involvement of JNK in the regulation of GF-induced G2/M arrest.

The transcriptional regulatory protein NF-κB has been shown to play important roles in the modulation of cellular proliferation, differentiation, inflammation, apoptosis, and cancer [Pahl, 1999; Sun and Andersson, 2002]. The RelA (p65) knockout mice is embryonically lethal as a result of extensive liver apoptosis suggesting the antiapoptotic activity of NF-κB [Barkett and Gilmore, 1999]. On the other hand, under certain circumstances, NF-κB can promote cell death evidenced by its cooperation with AP-1 to induce Fas ligand in T-cell [Kasibhatla et al., 1998]. The molecular mechanisms to determine the switch from an anti- to a pro-apoptotic role for NF-κB remained undefined. NF-κB can directly enhance tumorigenesis by inducing cellular proliferation through activation of proto-oncogene such as c-myc and cyclin D1 [Guttridge et al., 1999; Pahl, 1999]. Furthermore, the genes encoding relA(p65), c-Rel, p105, and p100 have all been reported to be associated with tumorigenesis of both solid and hematopoietic tumors [Rayet and Gelinas, 1999]. One report demonstrated that NF-κB was required for the HIF-1-dependent microtubule reorganization induction by the microtubule damaging agents [Jung et al., 2003]. Furthermore, microtubule disassembly induced by vinblastine activated the c-myc oncogene through NF-κB [Bourgarel-Rey et al., 2001]. All these findings suggested the potential roles of NF-κB involved in the GF-induced cell cycle regulation and apoptosis.

In this study, we test the effect of GF on the human leukemic and colon cancer cells and delineate its molecular mechanism. We demonstrated that different pathways are involved in the GF-induced G2/M arrest in colon and leukemic cell lines. We also, for the first time, demonstrated that NF-κB pathway is involved in the GF-induced G2/M arrest and apoptosis in HL-60 cells.

MATERIALS AND METHODS

Cell Culture and Chemical

Two human malignant cell lines (HL-60 and HT-29) were used in this study. HL-60 cells was developed from human myeloid leukemia cells [Collins et al., 1977]. HT-29 (HTB-38; American Type Culture Collection) originated from a human colon adenocarcinoma. The cells were grown in RPMI-1640 supplemented with 10% fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 μg/ml), and 0.3 mg/ml glutamine in a humidified incubator (37°C, 5% CO2). GF (Sigma Chemical Co., St. Louis, MO) was added at the indicated doses in 0.1% DMSO. For control specimens, the same volume of the 0.05% DMSO without GF was added.

Analysis of DNA Fragmentation

Analysis of DNA fragmentation was performed as previously described [Lin et al., 2001]. Briefly, the GF and DMSO-treated cells were seeded on 100-mm dishes. The DNA was extracted twice with equal volumes of phenol and once with chloroform-isoamyl alcohol (24:1 v:v), then precipitated with 0.1 volume of sodium acetate, pH 4.8, and 2.5 volumes of ethanol at −20°C overnight, and finally centrifuged at 13,000*g* for 1 h. Genomic DNA was quantitated, and equal amounts of DNA sample in each lane were electrophoresed in a 2% agarose gel. The DNA was visualized by ethidium bromide staining.

Flow Cytometry Analysis

Various GF solutions were prepared in a final concentration of 0.05% (v/v) DMSO. The cell cycle stages in the GF and DMSO-treated groups were measured by flow cytometry analysis as previously described [Lin et al., 2002]. Cells were harvested and stained with propidium iodide (50 μg/ml) (Sigma Chemical

Co.), and DNA content was measured using a FACScan laser flow cytometer analysis system (Becton-Dickinson, San Jose, CA); and 15,000 events were analyzed for each sample.

Western Analysis

Western blotting analysis was performed as described previously [Ho et al., 2005]. Briefly, cell lysates were prepared, electrotransferred, immunoblotted with antibodies, and then visualized by incubating with the colorigenic substrates (nitroblue tetrazolium, NBT, and 5-bromo-4-chloro-3-indolyl phosphate, BCIP) (Sigma Chemical Co.). The expression of GAPDH was used as the control for equal protein loading.

Immunoprecipitation and Cdc2 Kinase Activity Assay

The cdc2 kinase activity was determined as previously described [Ho et al., 2001]. Briefly, the protein lysate (100 μg in 0.5 ml of extraction buffer) was immunoprecipitated with 5 μg monoclonal anticyclin B1 antibody. The immuno-complexes were washed three times with the lysis buffer and once with kinase buffer containing 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, and 1 mM dithiothreitol. The beads were incubated with 50 μl of kinase reaction mixture containing 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 1 mM dithiothreitol, 10 μM ATP, 5 μCi of [γ-^{32}P] ATP, and 0.5 mg/ml of histone H1 for 15 min. The reaction was terminated by the addition of 20 μl of 4X Laemmli's sample buffer and boiling for 5 min. The ^{32}P-phosphorylated histone H1 was separated by 0.1% SDS, 10% polyacrylamide gel and determined by autoradiography using Kodak X-Omat film.

Electrophoretic Mobility Shift Assays (EMSA)

EMSA was determined as previously described [Lee et al., 2003]. Briefly, double-stranded DNA probe containing the NF-κB consensus oligonucleotide (5′-AGTTGAG-GGGACTTTCC-CAGG-3′) was labeled with [γ-^{32}P]ATP. The radiolabeled DNA (4 ng, 100,000 cpm) was incubated with nuclear extract in 15 μl of binding buffer (10 mM TRIS-HCl, pH 8.0, 1 mM EDTA, 10% glycerol, 200 mM NaCl, and 1 μg probe of DNA) on ice for 5 min. The samples were electrophoresed in a 5% polyacrylamide gel, dried on Whatman 3M paper and then exposed to Fuji X-ray films at −70°C.

Super-shift assay was performed as previously described [Kurozumi and Kojima, 2005] with some modification. Briefly, 2.5 μl of anti-p50 or anti-p65 antibodies (Santa Cruz Biotech, Santa Cruz, CA) were added to the sample for 60 min at 37°C. The radiolabeled NF-κB consensus oligonucleotide and 10 mM Tris (pH 7.5) 50 mM NaCl, 1 mM DTT, 1 mM EDTA, 5% glycerol, and 1 μg salmon sperm DNA were added to the sample for 20 min at room temperature. The sample then loaded onto a 4% polyacrylamide gel containing 50 mM Tris, 0.38 M glycine, 2 mM EDTA and subjected to electrophoresis. The DNA binding activity of each nuclear extract is presented as the amount of ^{32}P-labeled DNA-transcription factor complex detected by autoradiography.

RESULTS

GF-Induced G2/M Arrest and Apoptosis in HL-60 Cells but G2/M Arrest in HT-29 Cells

We first tested the cytotoxic effect of GF on HL-60 cells. As shown in Figure 1A, treatment of HL-60 cells with 30 to 60 μM GF for 8 h induced significant portion (70 to 80%) of cells in G2/M phase compared to 30% in control group suggesting an induction of G2/M arrest in HL-60 cells by GF. Treatment of HL-60 cells with 30 to 60 μM GF for 11 to 12 h induced significant portion of cells in sub G1 phase (20%) compared to control group suggesting an induction of apoptosis in HL-60 cells by GF. In contrast, treatment of HT-29 cells with 30 to 60 μM GF induced significant portion of cells in G2/M phase with maximum response at 12 h (50 to 80%). However, under similar treatment, no significant apoptotic response was noted (Fig. 1B). These findings suggested that GF-induced G2/M arrest in both leukemic and colon cancer cells but induced apoptotic response only in HL-60 cells.

Effect of NF-κB Inhibitor in the GF-Induced G2/M Arrest and Apoptosis in HL-60 and HT-29 Cells

Previous study demonstrated that N-tosyl-L-phenylalanine chloromethyl ketone (TPCK), a serine protease inhibitor, blocked taxol-induced apoptosis in MCF-7 human breast cancer cell [Huang et al., 1999]. It has been shown that TPCK inhibits phosphorylation of I-κB and, subsequently, activation of NF-κB [Jung et al., 1995]. We examined the possible involvement of NF-κB in the GF-induced G2/M arrest and

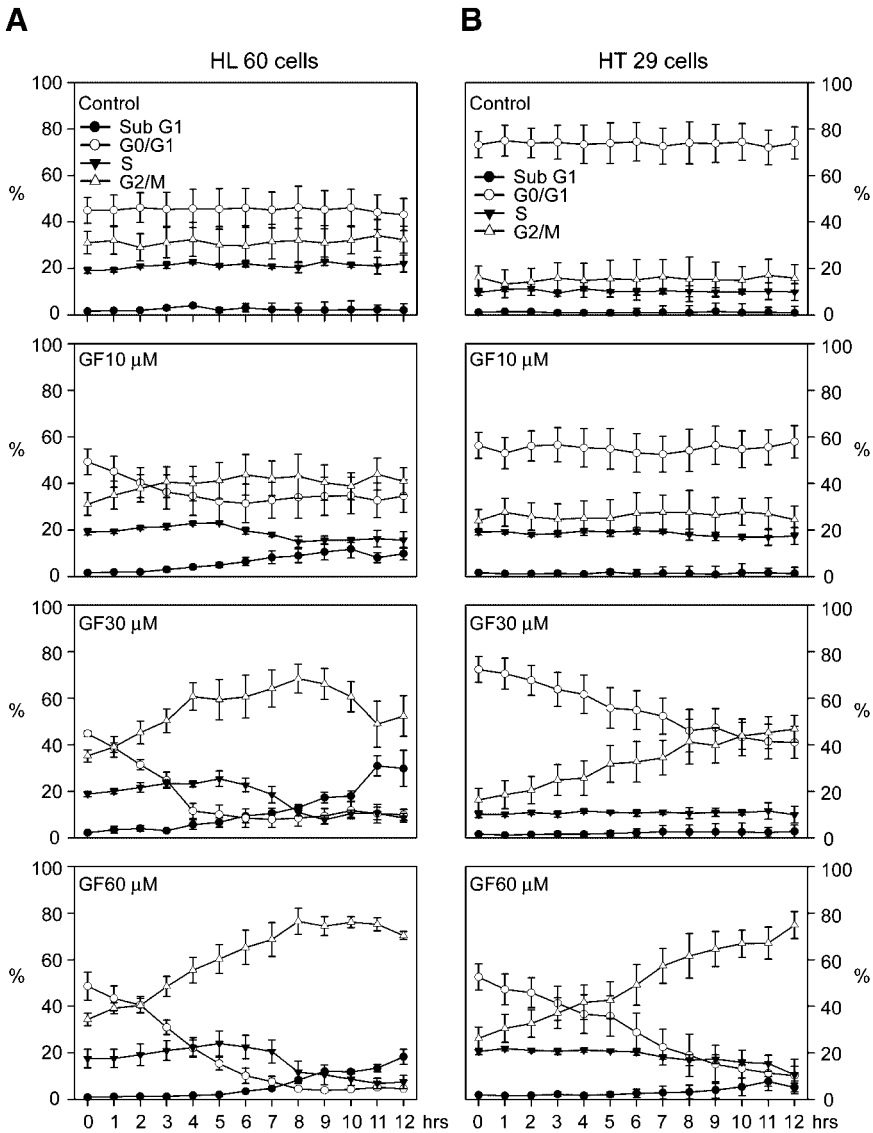

Fig. 1. Dose- and time-dependent response of GF-induced G2/M arrest and apoptosis in (**A**) HL-60 and (**B**) HT-29 cells. FACS analysis of DNA content was conducted after cells were harvested before incubation in culture media supplement with 10% FBS containing 0.05% DMSO (control) or GF (10, 30, 60 μM in 0.05% DMSO) for 0∼12 h. Percentage of cells in sub-G1, G0/G1, S, and G2/M phases of the cell cycle were determined using established CellFIT DNA analysis software. Three samples were analyzed in each group, and values were presented as means ± SE.

apoptosis in HL-60 and HT-29 cells. As shown in Figure 2A, the DNA fragmentation assay, pretreatment of 30 μM TPCK for 30 min blocked GF-induced apoptosis as well as nocodazole, another microtubule-damaging agent, -induced apoptosis in HL-60 cells. In contrast, no effect was observed in treating HT-29 cells (Fig. 2A, upper panel). It has been shown that apoptosis requries the activation of caspases [Thornberry and Lazebnik, 1998], we test the involvement of caspases in the GF-induced apoptosis in HL-60 cells. As shown in Figure 2B, treatment of HL-60 cell with 30 to 60 μM GF for 24 h resulted in the decreased staining of procaspase 3 with degradation of PARP, the substrate of caspase 3, suggesting the activation of caspase 3. To investigate the apoptotic pathways involved in the caspase 3 activation, we examined the changes of caspases 8 and 9 protein levels in the GF-treated HL-60 cells. Treatment of HL-60 cells with 30 to 60 μM GF for 24 h resulted in activation of caspases 8 and 9 evidenced by

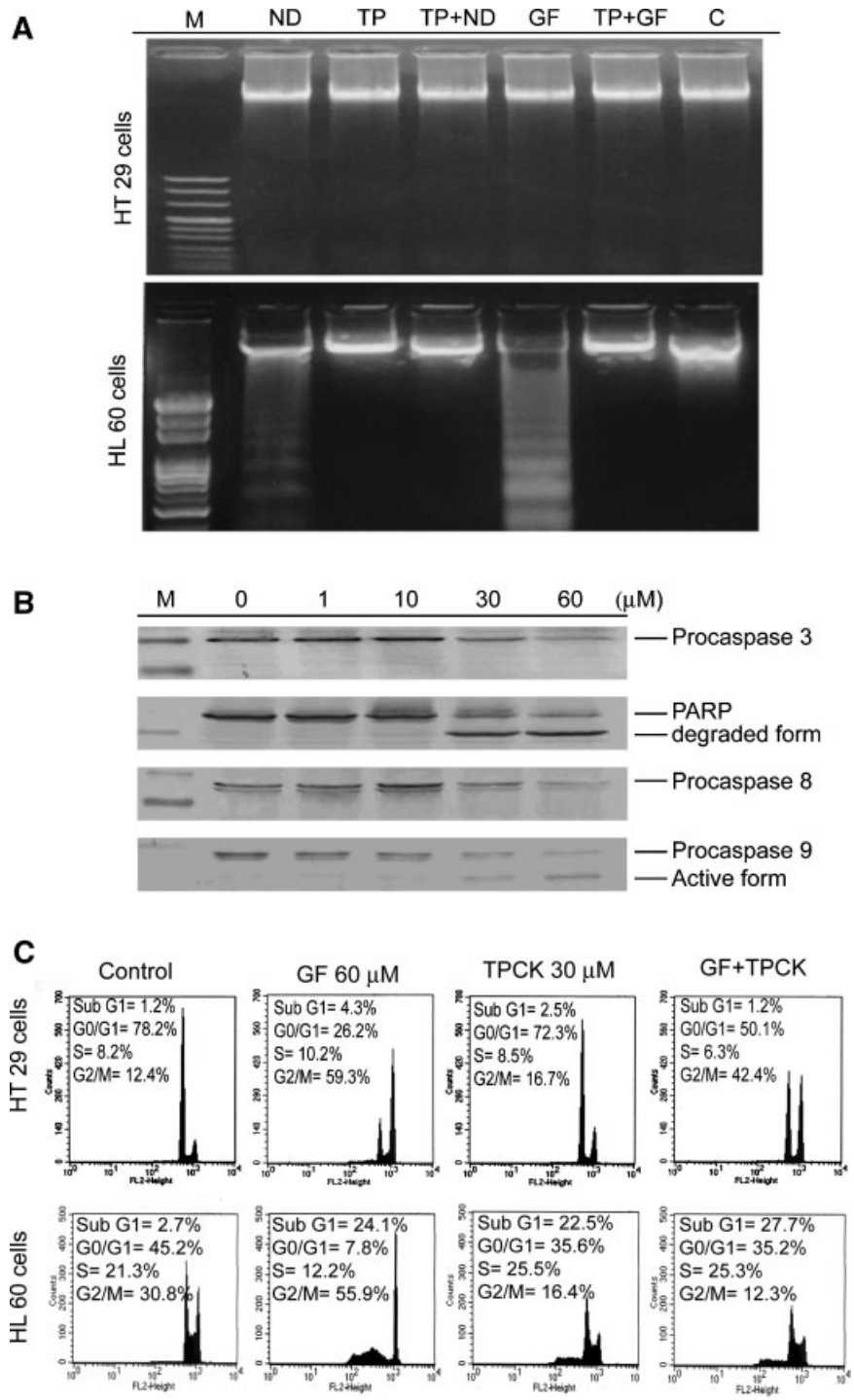

Fig. 2. **A**: Effects of TPCK on the GF and nocodazole-induced apoptosis in HT-29 and HL-60 cells. Cells were pretreated with TPCK 30 μM for 30 min then incubated with GF 30 μM or nocodazole 0.25 μM or control (0.05% DMSO) for 24 h. Extract DNA were prepared and subjected to DNA fragmentation analysis by 1.8% agarose gel electrophoresis. C, control; TP, TPCK; ND, nocodazole. **B**: Effects of GF on caspases, PARP protein levels. Protein extracts (100 μg per lane) were separated by SDS–PAGE. After electrophoresis, proteins were transferred onto Immobilion-P membranes, probed with proper dilution of specific antibodies, and then detected by using the NBT/BCIP system. **C**: Effects of TPCK on GF-induced G2/M cell cycle arrest in HT-29 and HL-60 cells. Cells were pretreated with TPCK 30 μM for 30 min before incubated with GF 60 μM or control (0.05% DMSO) for 12 h. Cells were harvested and flow cytometry assay were performed. Percentage of cells in sub-G1, G0/G1, S, and G2/M phases of the cell cycle were determined using established CellFIT DNA analysis software.

degradation of procaspase 8 and 9 as well as their cleavage products (Fig. 2B). As shown in Figure 2C, the flow cytometry assay, pretreatment of 30 μM TPCK for 30 min reversed GF-induced G2/M arrest. However, the effect was less prominent for HT-29 cells. These results suggested that NF-κB could be involved in the GF-induced G2/M arrest as well as apoptosis in HL-60 cells which caspases 3, 8, and 9 were involved.

GF-induced G2/M arrest in HL-60 is reversible. As shown in the time-dependent study of flow cytometry at Figure 3A,B, treatment of HL-60 cells with 60 μM GF at 3, 6, and 9 h resulted in G2/M population of 40, 60, and 60% respectively compared to 20% in the DMSO control group. Western blotting of the G2/M regulatory proteins demonstrated that Wee 1, cyclin E, and CDK4 proteins were downregulated but not cyclins A, B, CDK2, and cdc2 protein levels compared to DMSO control after GF treatment to HL-60 cells (Fig. 3A,B). To demonstrate whether GF-induced G2/M arrest in HL-60 cells is reversible, time-dependent

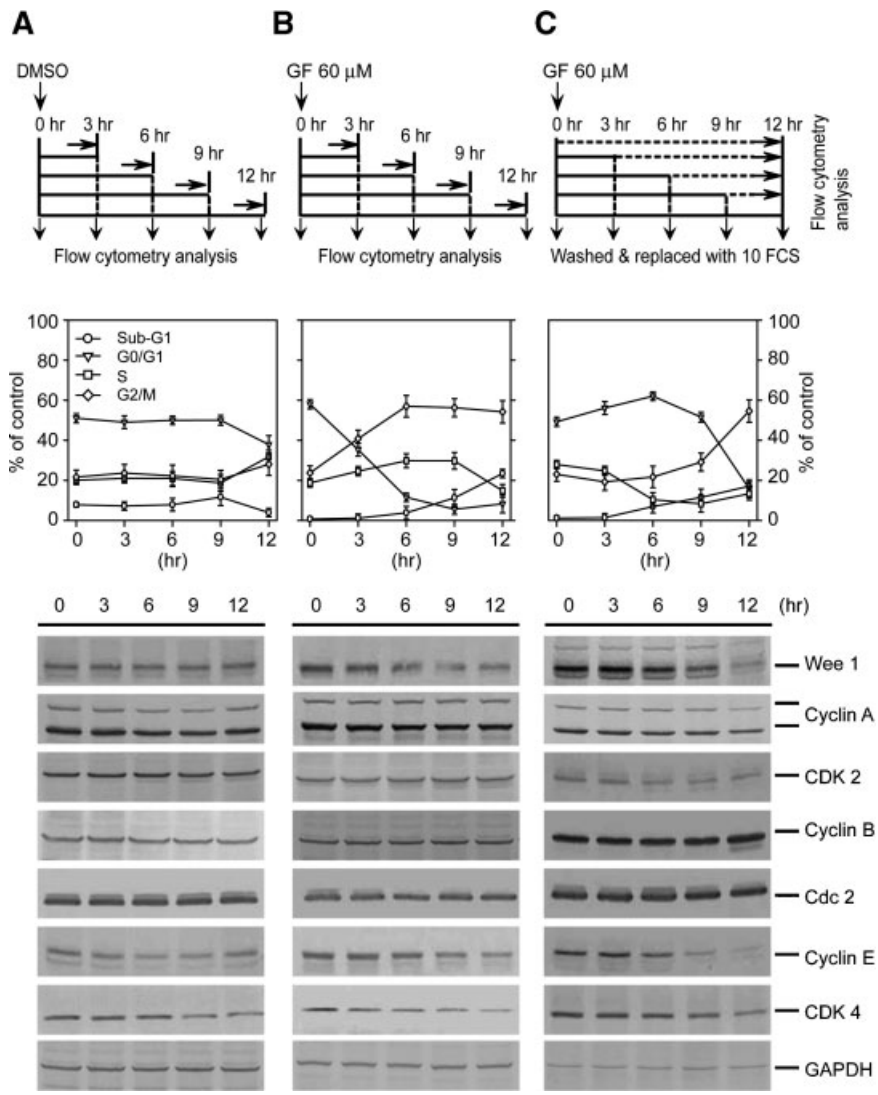

Fig. 3. Reversible effect of GF-induced G2/M cell cycle arrest in HL-60 cells. Cells were treated with (**A**) control (0.05% DMSO) or (**B**) 60 μM GF or (**C**) 60 μM GF and washed for the indicated time points, then harvest cells for flow cytometry assay (A–C, upper panels) or Western blotting analysis (A–C, lower panels). Percentage of cells in sub-G1, G0/G1, S, and G2/M phases of the cell cycle were determined using established CellFIT DNA analysis software. Three samples were analyzed in each group, and values were presented as means ± SE. Protein extracts (100 μg per lane) were separated by SDS–PAGE. After electrophoresis, proteins were transferred onto Immobilion-P membranes, probed with proper dilution of specific antibodies, and then detected by using the NBT/BCIP system. Membranes were also probed with GAPDH as loading control.

study of flow cytometry were performed as shown in Figure 3C. Treatment of HL-60 cells with 60 μM GF for 3, 6, and 9 h then washed the media with PBS and re-supplemented with fresh medium at 3, 6, and 9 h resulted in the decrease of G2/M population to 20, 25, and 30%, respectively. Under the same condition, the G2/M regulatory proteins such as Wee 1, cyclin E, and CDK4 showed no significant difference between pre-washed and washed HL-60 cells (Fig. 3B,C). These findings suggested that GF-induced G2/M arrest in HL-60 cells is reversible but not the G2/M regulatory protein expression.

Effect of GF on DNA-Binding Activity of NF-κB in HL-60 Cells

To test whether NF-κB activation mediated the GF-induced G2/M arrest in HL-60 cells, changes in the DNA-binding activity of transcription factor were assessed by EMSA. As shown in Figure 4A, GF treatment to HL-60 induced significant elevation of NF-κB DNA-binding activity started from 30 min and maintained elevation at 180 min after treatment. To further confirm the nature of the activated NF-κB-oligonucleotide complex in HL-60 cells, we performed super-shift assay. As shown in Figure 4B, the binding activity of the activated NF-κB-oligonucleotide complex with antibody to p65 subunits of NF-κB shifted to a higher molecular weight. To test whether TPCK could inhibit NF-κB activity in GF-treated HL-60 cells, we performed experiment as shown in Figure 4C. Pretreatment of 30 μM TPCK for 30 min blocked GF-induced NF-κB activation in HL-60 cells. These findings suggested that activation of p65 subunit of NF-κB mediated the GF-induced G2/M arrest in HL-60 cells. Arrow denoted un-specified bands which might be associated with TPCK treatment to HL-60 cells.

NF-κB Pathway Is Involved in the GF-Induced Bcl-2 Phosphorylation in HL-60 Cells

To further address upstream pathways associated with GF-induced G2/M arrest in HL-60 cells, we performed immunoblotting assays. As shown in Figure 5A, treatment of 30 μM GF to HL-60 cells induced JNK phosphorylation started from 30 min and peaked at 60 min in a time course study. However, no significant change of ERK or p38 MAPK was noted.

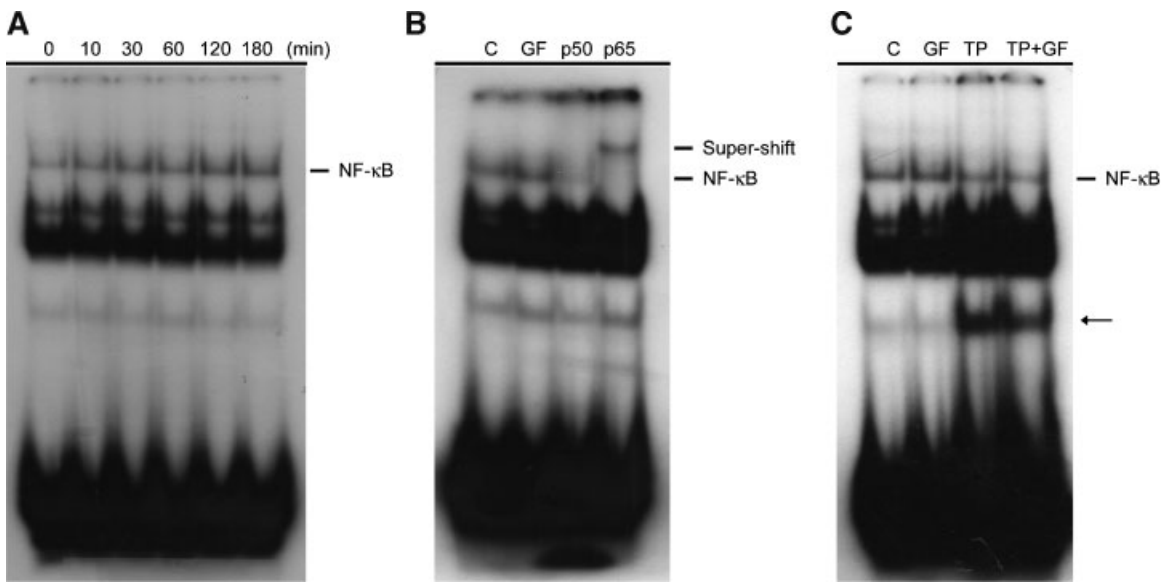

Fig. 4. Effect of GF on DNA-binding activity of NF-κB in HL-60 cells. **A**: Time-dependent activation of NF-κB in nuclear extracts of HL-60 cells stimulated with GF. Cells were treated with GF 30 μM for the indicated time points. Nuclear extracts was prepared and DNA-protein binding activity were determined by electrophoresis mobility shift assay. **B**: Cells were treated with GF 30 μM or control (0.05% DMSO) for 30 min and nuclear extracts were prepared. Super-shift assay to the DNA complex was verified by anti-p50 or anti-p65 antibodies. **C**: TPCK attenuated GF-induced NF-κB activation in nuclear extracts of HL-60 cells. Cells were pretreated with 30 μM TPCK for 30 min before incubated with 30 μM GF or control (0.05% DMSO) for 30 min, and then nuclear extracts were prepared. The DNA-protein binding activity in nuclear extracts by electrophoresis mobility shift assay. C, control; TP, TPCK; p50 and p65, antibodies for p50 and p65 subunits of NF-κB, respectively. Arrow denoted unspecified bands.

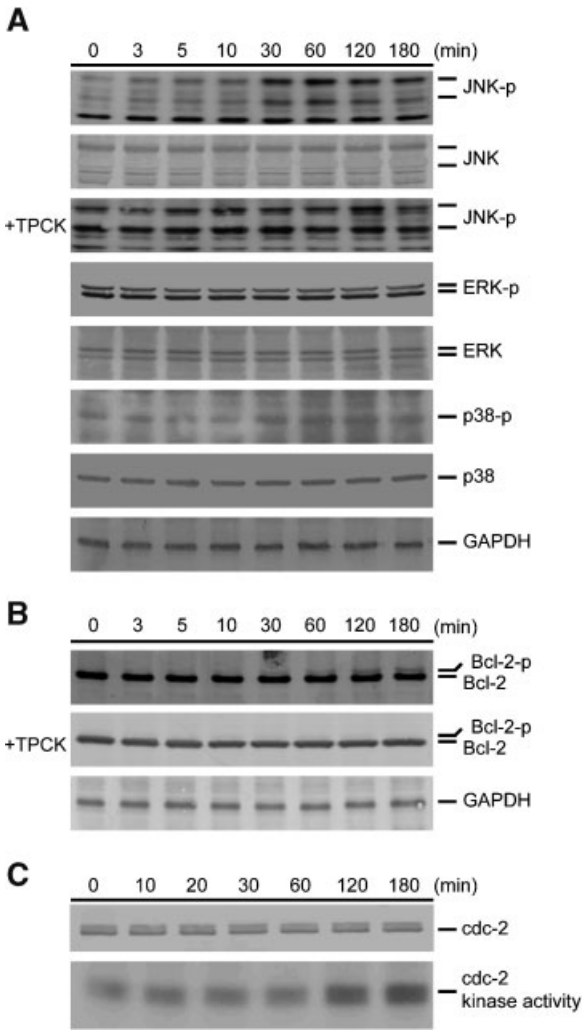

Fig. 5. Effect of JNK and NF-κB pathways in the GF-induced Bcl-2 phosphorylation and cdc-2 kinase activation in HL-60 cells. **A**: Effect of GF-induced MAPK phosphorylation and TPCK inhibition effect in HL-60 cells, time-dependent study. Cells were treated with 30 μM GF for various time intervals. Whole cell lysates were prepared, then Western blot analysis using specific antibodies directed against the phosphorylation or total protein. For the inhibitor study, cells were pretreated with 30 μM TPCK (+ TPCK) before incubated with 30 μM GF for the indicated time points. Membrane was also probed with GAPDH as loading control. **B**: Effect of GF-induced Bcl-2 phosphorylation and TPCK inhibition effect in HL-60 cells, time-dependent study. Whole cell lysates were prepared and subjected to Western blot analysis using specific antibodies directed against the phosphorylation or total protein. For the inhibitor study, cells were pretreated with 30 çM TPCK (+ TPCK) before incubated with 30 μM GF for the indicated time points. Membrane was also probed with GAPDH as loading control. **C**: Effect of cdc-2 kinase activation in the GF treatment to HL-60 cells. Cells were treated with 30 μM GF for various time intervals. Whole cell lysates were prepared, then cdc-2 kinase assay (upper panel) and Western blot analysis of cdc-2 protein (lower panel).

Pretreatment of 30 μM TPCK for 30 min did not block GF-induced JNK activation. Under similar condition, phosphorylation of Bcl-2 was observed after 120 to 180 min of GF treatment. Pretreatment of 30 μM TPCK for 30 min blocked GF-induced Bcl-2 phosphorylation (Fig. 5B). It has been demonstrated that cdc-2 could activate Bcl-2 phosphorylation associated with okadaic acid-induced G2/M arrest in HL-60 cells [Furukawa et al., 2000]. As shown in Figure 5C, treatment of HL-60 cells with 30 μM GF induced cdc-2 kinase activity started after 120 min of treatment without change in cdc-2 protein expression. These findings suggested that NF-κB but not JNK pathway is involved in the Bcl-2 phosphorylation, which might be further involved cdc-2 activation in GF-induced cytotoxicity in HL-60 cells.

DISCUSSION

The present study was undertaken to examine the molecular mechanisms involved in the GF-induced G2/M cell cycle arrest and apoptosis in human leukemic and colon cancer cells. Our studies demonstrated that GF at concentrations of 30 to 60 μM induced mitotic arrest and apoptosis in HL-60 cell but only mitotic arrest in HT-29 cells. The GF-induced G2/M arrest in HL-60 is reversible. NF-κB signaling pathway is involved in the GF-induced cytotoxicity in HL-60 cells with subsequently cdc-2 activation and Bcl-2 phosphorylation. To our knowledge, this is the first demonstration to elucidate upstream pathways in GF-induced mitotic arrest in HL-60 cells.

We noted that in Figure 1A, the percentage of sub G1 cells decreased with the increase of GF from 30 μM to 60 μM. We demonstrated in our report that low dose loratadine induced apoptosis while high dose induced G2/M arrest in COLO 205 cells [Chen et al., 2006]. Treatment of checkpoint kinase 1 (Chk1) inhibitor (SB-218078) released COLO 205 cells from G2/M arrest and resulted in the induction of apoptosis. The findings are consistent with our results, which show low dose GF induced more apoptotic response than the high dose GF treatment suggesting that high dose GF induces higher degree of G2/M arrest, which might suppress the GF-induced apoptotic pathways. Similar findings were noted with knockdown of Chk1, Wee1, and Myt1 by RNA interference, which resulted in abrogation of adriamycin or

paclitaxel-mediated G2 checkpoint and subsequently induction of apoptosis in human cells [Wang et al., 2004; Ren et al., 2005; Xiao et al., 2005].

The antifungal drug GF inhibited mitosis by affecting microtubule dynamics [Panda et al., 2005]. But its underlying mechanism to induce G2/M arrest and apoptosis in human cancer cells remained obscure [Ho et al., 2001]. Our results demonstrated that TPCK inhibitable NF-κB pathway is involved in the GF-induced apoptosis in HL-60 cells. The finding is consistent with the report that NF-κB /I-κB signaling pathway contributes to the mediation of antimicrotubule vinca alkaloid-induced apoptosis in human breast cancer BCap37 cells and human epidermoid tumor KB cells [Huang et al., 2004]. Our results demonstrated that GF-induce NF-κB and JNK activation and subsequently Bcl-2 phosphorylation in HL-60 cells. Suppression of NF-κB but not JNK activity by TPCK reversed GF-induced Bcl-2 phosphorylation and apoptosis in HL-60 cells suggesting involvement of NF-κB and Bcl-2 pathways in GF-induced apoptosis in HL-60 cells. The finding is consistent with the report that vinblastine mediated apoptosis through Bcl-2/Bcl-X(L) phosphorylation [Fan et al., 2000].

As to the molecular mechanisms of GF-induced G2/M arrest in HL-60 and HT-29 cells, we demonstrated that TPCK inhibitable NF-κB pathway is involved in HL-60 but not HT-29 cells. How NF-κB signaling regulates GF-induced G2/M arrest remained obscured. It has been documented that microtubule-stabilization agents upregulated maturation promoting factor (cyclin B1-cdc2 complex) activity [Ling et al., 1998; Blagosklonny and Fojo, 1999]. Our results demonstrated that cdc2 kinase activity was marked elevated after 2 h of GF treatment to HL-60 cells without change of cdc-2 protein level. Previous reports suggested that loss of cdc-2 activity was the key event in promoting exit from the mitosis [Tan et al., 2005; Bergstralh and Ting, 2006]. It has been reported that EB virus latent membrane protein-induced G2/M arrest of nasopharyngeal carcinoma cells through NF-κB activation and cdc2/cyclin B regulation [Deng et al., 2003]. We proposed that GF-induced NF-κB activation and mediated the sustained activity of cdc-2 and resulted in blockage of mitotic exit and G2/M arrest.

Our previous report demonstrated that GF potentiated nocodazole-induced antitumor effect in a nude mice xenograft model [Ho et al., 2001]. No sign of gross and microscopic toxicity was observed in the GF treatment group as compared to control. The present study showed the reversibility of GF-induced G2/M arrest and apoptosis to HL-60 cells consistent with the low toxicity of GF. Further studies are required to clarify the role of GF as a safe and effective agent to enhance chemotherapy response in treating cancer.

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