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Inhibition of JNK2 and JNK3 by JNK inhibitor IX induces prometaphase arrest-dependent apoptotic cell death in human Jurkat T cells

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ABSTRACT

Exposure of human Jurkat T cells to JNK inhibitor IX (JNKi), targeting JNK2 and JNK3, caused apoptotic DNA fragmentation along with G_2/M arrest, phosphorylation of Bcl-2, Mcl-1, and Bim, $\Delta\psi$m loss, and activation of Bak and caspase cascade. These JNKi-induced apoptotic events were abrogated by Bcl-2 overexpression, whereas G_2/M arrest, cyclin B1 up-regulation, Cdk1 activation, and phosphorylation of Bcl-2 family proteins were sustained. In the concomitant presence of the G_1/S blocking agent aphidicolin and JNKi, the cells underwent G_1/S arrest and failed to induce all apoptotic events. The JNKi-induced phosphorylation of Bcl-2 family proteins and mitochondrial apoptotic events were suppressed by the Cdk1 inhibitor. Immunofluorescence microscopic analysis revealed that mitotic spindle defect and prometaphase arrest were the underlying factors for the G_2/M arrest. These results demonstrate that JNKi-induced mitochondrial apoptosis was caused by microtubule damage-mediated prometaphase arrest, prolonged Cdk1 activation, and phosphorylation of Bcl-2 family proteins in Jurkat T cells.

1. Introduction

The eukaryotic cell cycle progression is controlled by the sequential activation and inactivation of a series of cyclin-dependent kinases (Cdks) [1]. To ensure the fidelity of cell division, passage through a series of cell cycle checkpoints, which act as molecular breaks, to verify the accuracy of cell cycle progression is required [2]. Three checkpoints are involved in the cell cycle control system: (1) the G_1 checkpoint, which functions in the G_1 phase to confirm that the environment is favorable for cell proliferation before committing to the S phase, (2) the G_2 checkpoint, which functions in the G_2 phase to prevent entry into mitosis until damaged DNA is repaired and DNA replication is completed, and (3) the mitotic spindle assembly checkpoint, which functions during mitosis and ensures proper attachment of the replicated chromosomes to the mitotic spindles [3–5]. A combination of several distinct mechanisms operates to negatively regulate Cdk activity during G_1 or G_2 checkpoint activation. These include increased cyclin destruction, decreased cyclin gene expression, Cdk inhibition by Cdk inhibitors (CKIs; $p21^{CIP1/WAF1}$, $p27^{KIP1}$, and $p16^{INK4A}$) that bind and inactivate Cdk/cyclin complexes, and Cdc25 inactivation. However, when the mitotic spindle assembly checkpoint becomes activated, Cdk1/cyclin B is kept in an active state by inhibiting the role of the anaphase-promoting complex in order to prevent the onset of anaphase.

The cell cycle regulation system is influenced by extracellular signaling pathways, where mitogen-activated protein kinases (MAPKs) play a key role in modulating Cdks, cyclins, and/or CKIs to determine the passage through checkpoints [6,7]. Mammalian MAPKs consist of three major subgroups, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK. The ERK activity, resulting from growth factor-mediated or adhesive signals-mediated activation of the Ras/ERK pathway, has been shown to phosphorylate transcription factors AP1 and ETS, which subsequently induce cyclin D gene expression. ERK-mediated up-regulation of $p21^{CIP1/WAF1}$ is known to promote assembly of cyclin D–Cdk4/6 kinase complexes that phosphorylate pRB, leading to the activation of the transcription factor E_2F that regulates the transcription of genes required for G_1/S transition. Additionally, $p27^{KIP1}$ phosphorylation by ERK results in its degradation and release of active cyclin E/Cdk2, and thus allows the cells to enter the S phase, indicating that ERK positively modulates the passage through the G_1 checkpoint.

Stress-activated MAPK pathways are more likely involved in the negative modulation of the cell cycle; the p38 MAPK pathway inhibits the phosphorylation of AP1 and ETS required for cyclin D synthesis, leading to cell cycle arrest, and the JNK pathway

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suppresses cell cycle progression by attenuating E_2F-dependent transcription [6]. In addition, numerous studies have reported that the JNK pathway is associated with many forms of stress-induced apoptotic cell death [8]. Under certain conditions, however, JNK is known to support cell survival and cell proliferation, and inhibits apoptosis. For examples, inhibition of JNK using a pan JNK inhibitor SP600125 (10–20 μM) or JNK1/2-specific siRNA results in G_2/M arrest and apoptosis in murine and human B-lymphomas which constitutively express high levels of active JNK [9]. In several tumor cell lines after treatment with SP600125 (20–40 μM), abnormal microtubule dynamics, G_2/M arrest, polyploidy, and apoptosis induction are detected [10,11]. These previous results suggest that JNK activity might also be necessary to progress through the G_2/M phase and maintain diploidy via a role in mitotic spindle assembly, and that the JNK inhibitor-mediated G_2/M arrest observed might be due to the operation of the mitotic spindle assembly checkpoint.

Recently, the pharmacological use of the JNK inhibitor has been proposed as a promising antitumor agent that induces G_2/M arrest and apoptosis of tumor cells [9,10]. However, because treatment with >10 μM SP600125 has been shown to inhibit cyclin A/Cdk activity [12], and cyclin B/Cdk1 activity [13] in addition to JNK1, JNK2, and JNK3 activities, the possibility cannot be excluded that the cellular responses detected following treatment with greater than 10 μM SP600125 were due to some nonspecific effects of SP600125. Furthermore, the death mechanism underlying JNK inhibitor-induced apoptosis in tumor cells remains obscure.

In this study, in order to examine how cellular JNK inhibition by a validated JNK inhibitor (JNK inhibitor IX, JNKi), which acts as the ATP binding site-targeting inhibitor of JNK2 and JNK3 [14], cause apoptotic cell death, we investigated the apoptogenic mechanism of JNKi using human Jurkat T cell clone stably transfected with an empty vector (JT/Neo) or a Bcl-2 expression vector (JT/Bcl-2). In addition, to examine the dependency of JNKi-induced apoptotic events on G_2/M arrest, the effect of aphidicolin (APC), which is known to arrest cell cycle progression at the G_1/S boundary [15], on the JNKi-induced apoptotic events was investigated.

2. Materials and methods

2.1. Reagents, antibodies, and cells

APC, 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$), and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma Chemical (St. Louis, MO). An ECL Western blot kit was purchased from Amersham (Arlington Heights, IL), and the Immobilon-P membrane was obtained from Millipore Corporation (Bedford, MA). The anti-caspase-3 antibody was purchased from Pharmingen (San Diego, CA), and the anti-poly (ADP-ribose) polymerase (PARP), anti-Bax, anti-Bim, anti-Bcl-2, anti-Bcl-xL, anti-Mcl-1, anti-Cdk1, anti-cyclin B1, anti-Aurora A kinase (ARK1), anti-histone H1, anti-p-histone H3 (Ser-10), anti-histone H3, and anti-lamin B antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-caspase-9, anti-p-Cdk1 (Tyr-15), anti-p-Cdk1 (Thr-161), anti-p-Cdc25C (Thr-48), anti-Cdc25C, anti-p-Bcl-2 (Thr-56), anti-p-Bcl-2 (Ser-70), anti-p-Mcl-1 (Ser-159/Thr-163), anti-p-ARK1 (Thr-288), and anti-α-tubulin antibodies were purchased from Cell Signaling Technology (Beverly, MA), and the anti-p-histone H1 antibody was purchased from Upstate Biotechnology (Lake Placid, NY). The anti-Bak (Ab-1) and anti-Bax (6A7) antibodies were obtained from Calbiochem (San Diego, CA). The anti-p-Bcl-xL (Ser-62) antibody was obtained from Invitrogen (Carlsbad, CA). The Cdk1 inhibitor RO3306 was purchased from Tocris Bioscience (Ellisville, MO), and the ARK1 inhibitor MLN8237 and Aurora B kinase inhibitor AZD1152-HQPA were obtained from Selleck (Houston, TX). The JNK inhibitor IX (JNKi) was obtained from Santa Cruz Biotechnology and the p38 MAPK inhibitor SB202190 was purchased from Biomol (Plymouth Meeting, PA). The human acute leukemia Jurkat T cell clone, transfected with a *Bcl-2* expression vector (JT/Bcl-2) or with an empty vector (JT/Neo) was kindly provided by Dr. Dennis Taub (Gerontology Research Center, NIA/NIH, Baltimore, MD). Both JT/Neo cells and JT/Bcl-2 cells were maintained in RPMI 1640 medium containing 10% FBS, 20 mM HEPES (pH 7.0), 5×10^{-5} M β-mercaptoethanol, 100 μg/ml gentamicin, and 400 μg/ml G418 (A.G. Scientific Inc., San Diego, CA).

2.2. Flow cytometric analysis

Flow cytometric analysis to measure cell cycle profile of Jurkat T cells exposed to JNKi was performed as described elsewhere [16]. The extent of necrosis was detected using an Annexin V-FITC apoptosis kit as previously described [16]. The change in the mitochondrial membrane potential ($\Delta\psi m$) following JNKi treatment was measured after staining with $DiOC_6$ [17]. Activation of Bak and Bax following JNKi treatment was measured as previously described [17].

2.3. Immunofluorescence microscopy

Immunostaining of Jurkat T cells treated with JNKi was performed as previously described [17].

2.4. Preparation of cell lysates and Western blot analysis

Cell lysates were prepared as described elsewhere [16]. An equivalent amount of protein lysate (20 μg) was electrophoresed on a 4–12% NuPAGE gradient gel and then electrotransferred to an Immobilon-P membrane. Protein detection was performed using an ECL Western blot kit according to the manufacturer's instructions. Densitometry was performed using ImageQuant TL software (Amersham, Arlington Heights, IL). The arbitrary densitometric units for each protein of interest were normalized to the densitometric units for α-tubulin.

2.5. Statistical analysis

Unless otherwise indicated, each result in this article is representative of at least three separate experiments. The values are expressed as the means ± standard deviation of these experiments. Statistical significance was calculated using Student's *t*-test. *P* values <0.05 were considered to be significant.

3. Results and discussion

3.1. Apoptogenic effect of JNKi on human Jurkat T cell clones JT/Neo and JT/Bcl-2

Since the antiapoptotic role of Bcl-2 is known to prevent caspase-3 activation by blocking cytochrome c release from the mitochondria [18], we decided to take advantage of this antiapoptotic role of Bcl-2 to examine whether the Bcl-2-sensitive mitochondrial apoptotic pathway is a key mediator of the cytotoxicity of JNKi. In this context, the cytotoxic effect of JNKi (25–50 nM) was compared using JT/Neo and JT/Bcl-2 cells. Results from the MTT assay showed that the viabilities of JT/Neo cells exposed to 25 nM and 50 nM JNKi for 20 h were 80.0% and 33.2%, respectively, whereas those of JT/Bcl-2 cells were 98.0% and 81.5%, respectively (Fig. 1A). In addition, JNKi appeared to induce DNA fragmentation in JT/Neo cells in a dose-dependent manner, but it failed to induce DNA fragmentation in JT/Bcl-2 cells (Fig. 1B). Similarly, after treatment with JNKi at

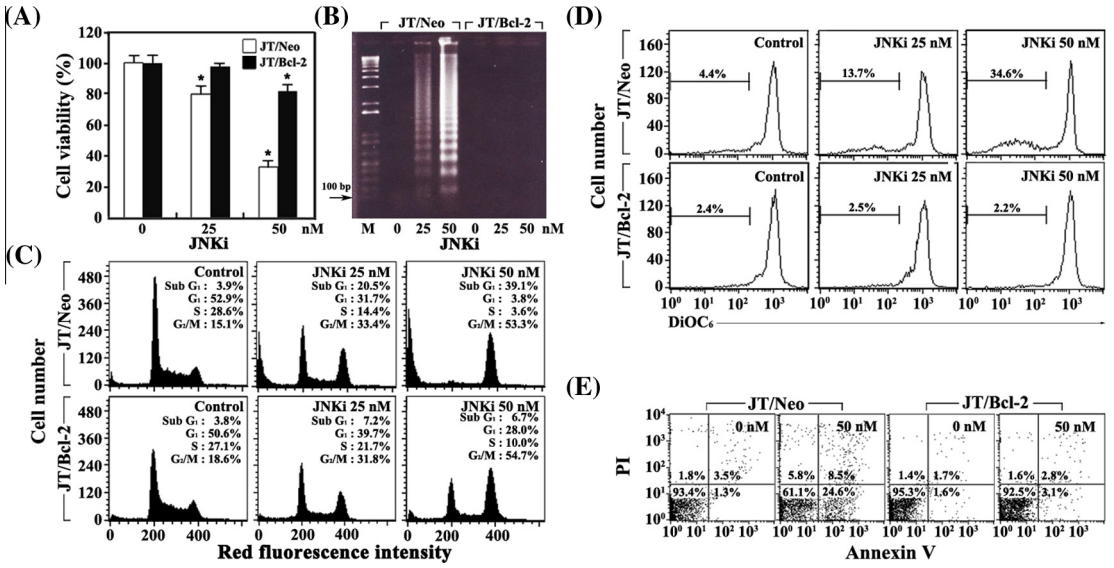

Fig. 1. Effect of JNKi on cell viability (A), apoptotic DNA fragmentation (B), cell cycle distribution (C), $\Delta\psi$m loss (D), and apoptotic cell death (E) in Jurkat T cell clone transfected with an empty vector (JT/Neo) and Jurkat T cell clone transfected with a *Bcl-2*-expression vector (JT/Bcl-2). To investigate cell viability, individual cells (6×10^4 cells/well) were incubated in the indicated doses of JNKi in a 96-well plate for 20 h, and for the final 4 h, each cell was treated with MTT to assess cell viability [16]. Each value is expressed as the mean ± SD ($n = 3$; three replicates per independent experiment). $^*P < 0.05$ compared to the control. Equivalent cultures were prepared and harvested to analyze apoptotic DNA fragmentation by Triton X-100 lysis methods using 1.2% agarose gel electrophoresis [16]. Cell cycle distribution, $\Delta\psi$m loss, and apoptotic cell death were determined by flow cytometric analysis as described in Section 2. A representative result is presented, and two additional experiments yielded similar results.

concentrations of 25 nM and 50 nM, JT/Neo cells exhibited an enhanced proportion of apoptotic sub-G_1 cells to the levels of 20.5% and 39.1%, respectively, whereas no enhancement in the proportion of apoptotic sub-G_1 cells was detected in JT/Bcl-2 cells (Fig. 1C). Under these conditions, the proportion of G_2/M cells was significantly enhanced in both JT/Neo and JT/Bcl-2 cells following JNKi treatment; however, there was no increase in the proportion of G_1-S cells in either cell type. When the loss of $\Delta\psi$m in cells treated with JNKi was measured by $DiOC_6$ staining, the percentage of JT/Neo cells that displayed decreased fluorescence following treatment with JNKi at concentrations of 25 nM and 50 nM were 13.7%, and 34.6%, respectively (Fig. 1D). In contrast, the JNKi-induced $\Delta\psi$m loss was completely abrogated in JT/Bcl-2 cells. Analysis by Annexin V-FITC and PI staining of JT/Neo cells treated with 50 nM JNKi for 20 h revealed that the number of early apoptotic cells stained only with Annexin V-FITC was enhanced, but late apoptotic cells stained with both Annexin V-FITC and PI, as well as necrotic cells stained only with PI were rarely detected (Fig. 1E), confirming that JNKi-induced apoptosis was not accompanied by necrosis.

These results indicate that the cytotoxicity of JNKi (25–50 nM) was due to a cytostatic effect mediated by G_2/M arrest, and apoptosis mediated by $\Delta\psi$m loss that was blocked by Bcl-2 overexpression in Jurkat T cells. Since JNKi is known to target JNK2 and JNK3 [14], these results suggest that the G_2/M arrest occurred prior to the induction of mitochondrial damage and resultant apoptosis, and was provoked via a JNK2 and/or JNK3-governed mechanism insensitive to the anti-apoptotic effect of Bcl-2.

3.2. Comparison of Cdk1 activation, Bcl-2 phosphorylation, Mcl-1 phosphorylation, Bim phosphorylation, Bak activation, caspase cascade activation, and cellular microtubule network in JT/Neo and JT/Bcl-2 cells following JNKi treatment

Previous studies have shown that the phosphorylation of Bcl-2 family proteins, such as Bcl-2, Bcl-xL, Mcl-1, and Bim by Cdk1 couples the microtubule inhibitor-induced mitotic arrest to apoptotic cell death [17,19–21]. The phosphorylation of Bcl-2 is known to cause its conformational changes that inactivate the anti-apoptotic function of Bcl-2, leading to the activation of Bax and/or Bak to provoke mitochondrial cytochrome *c* release [21,22]. Therefore, we decided to examine whether Cdk1 activation and the phosphorylation of Bcl-2 family proteins occurred upstream of the JNKi-induced mitochondrial apoptotic events.

The levels of Cdk1 phosphorylated at Tyr-15 were reduced, the levels of Cdk1 phosphorylated at Thr-161 were elevated, the expression levels of cyclin B1 were up-regulated, and total Cdk1 expression levels remained relatively constant in JT/Neo cells and JT/Bcl-2 cells treated with JNKi (Fig. 2A). Under these conditions, histone H1 phosphorylation, which is catalyzed by Cdk1 during G_2/M phase [23], was enhanced in both cell types. The phosphorylation of histone H3 at Ser-10 by Aurora B kinase and auto-phosphorylation of Aurora A kinase (ARK1) at Thr-288, which are known to be mediated by Cdk1 during the G_2/M phase [17], were also enhanced in both cell types. The phosphorylation of Cdc25C at Thr-48, representing the necessary activation step for Cdk1 dephosphorylation at Tyr-15, appeared to increase in both cell types. Current results indicated that Cdk1 was activated and its enzymatic activity was sustained during JNKi-induced mitotic arrest.

Bcl-2 phosphorylation at Thr-56 and Ser-70, Mcl-1 phosphorylation at Ser-159 and/or Thr-163, and Bim (Bim_{EL} and Bim_L) phosphorylation, as shown by their phosphorylation-induced reduction in mobility during SDS–polyacrylamide gel electrophoresis, were elevated in both cell types following JNKi treatment (Fig. 2B). However, Bcl-xL phosphorylation was not detected (data not shown). Although the expression levels of Bcl-2, Bcl-xL, Bad, Bak, Bax, and Bid remained relatively constant following JNKi treatment, the Mcl-1 expression level was markedly reduced, possibly due to its phosphorylation and resultant degradation by the proteasome system [20].

In addition, JNKi-induced Bak activation, as assessed using an anti-Bak (Ab-1) antibody that specifically recognizes the conformationally active Bak protein [17], was detected in JT/Neo cells but not in JT/Bcl-2 cells (Fig. 2C). In contrast, Bax activation was not detected in either cell type (data not shown). In accordance with

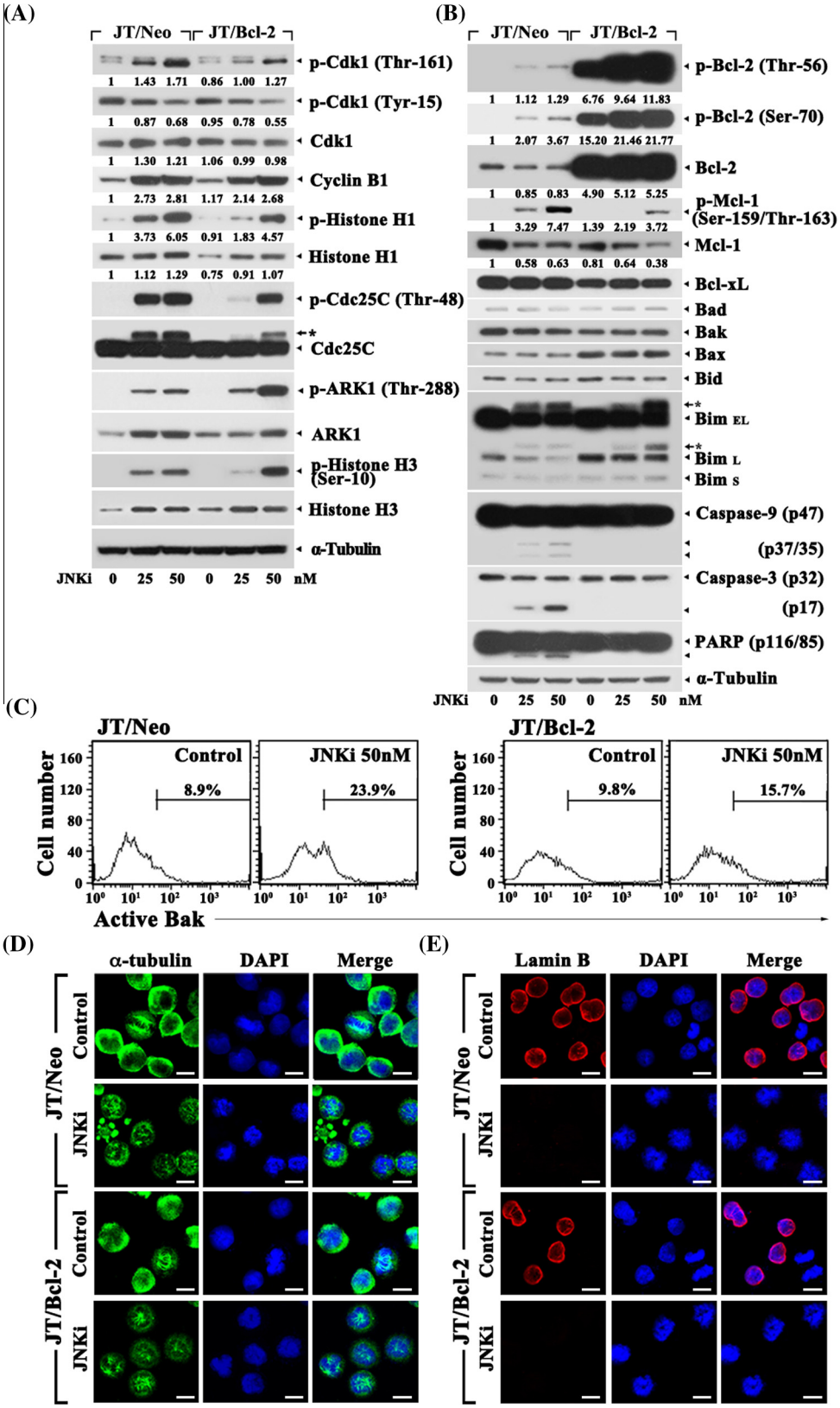

Fig. 2. Western blot analysis of phosphorylated Cdk1 (Thr-161 and Tyr-15), Cdk1, cyclin B1, phosphorylated histone H1, histone H1, phosphorylated Cdc25C (Thr-48), Cdc25C, phosphorylated ARK1 (Thr-288), ARK1, phosphorylated histone H3 (Ser-10), histone H3, and α-tubulin (A), phosphorylated Bcl-2 (Thr-56 and Ser-70), Bcl-2, phosphorylated Mcl-1 (Ser159/Thr-163), Mcl-1, Bcl-xL, Bad, Bak, Bax, reduction in the electrophoretic mobility of the Bim isoforms (Bim_{EL} and Bim_L), caspase-3 activation, PARP cleavage, and α-tubulin (B), flow cytometric analysis of Bak activation (C), and immunostaining analysis of JNKi-induced alteration in the microtubule organization (D) and nuclear envelope (E) in JT/Neo and JT/Bcl-2 cells following treatment with JNKi. The cells were treated with the indicated concentrations of JNKi for 20 h. Symbol: ←*, phosphorylated form of Cdc25C. A representative result is presented, and two additional experiments yielded similar results.

JNKi-induced Bak activation, caspase-9 activation, caspase-3 activation, and PARP cleavage were detected in JT/Neo cells, whereas these apoptotic events were completely abrogated in JT/Bcl-2 cells. These results demonstrated that the JNKi-induced apoptosis of Jurkat T cells was accompanied by Cdk1 activation, phosphorylation of Bcl-2, Mcl-1, and Bim, Bak activation, and activation of caspase-9 and -3, leading to PARP cleavage. The present results also indicated that Cdk1 activation and phosphorylation of Bcl-2, Mcl-1, and Bim occurred upstream of Bcl-2-sensitive Bak activation, and caspase cascade activation.

Two different molecular mechanisms, the G_2/M DNA damage checkpoint pathway and the mitotic spindle checkpoint pathway, are associated with chemotherapy-mediated G_2/M arrest accompanying apoptosis in tumor cells [3–5]. Following exposure to DNA-damaging drugs, cells can be arrested at the G_2/M DNA damage checkpoint by inactivating Cdk1/cyclin B. When cells fail to align all chromosomes on the metaphase plate following treatment with microtubule-damaging drugs, the mitotic spindle checkpoint becomes activated to prevent the onset of anaphase and Cdk1/cyclin B is kept in an active state by inhibiting the role of the anaphase promoting complex. Thus, we could exclude the contribution of the G_2/M DNA damage checkpoint to JNKi-induced G_2/M arrest and apoptosis because the Cdk1 activity was sustained during the G_2/M arrest by JNKi.

To confirm whether the JNKi-induced G_2/M arrest accompanies the mitotic spindle defect, the effect of JNKi on the organization of the microtubule network was investigated by immunofluorescence microscopy using an anti-α-tubulin antibody. After treatment with 50 nM JNKi for 20 h, both JT/Neo and JT/Bcl-2 cells exhibited an aberrant bipolar array of microtubules (Fig. 2D). Additionally, DAPI staining and immunostaining using anti-lamin B antibody revealed that most of the chromosomes in both cell types treated with JNKi were not properly aligned at the equator of the mitotic spindle and appeared to possess no nuclear envelope (Fig. 2E). Prometaphase begins with the breakdown of the nuclear envelope and continues until the sister chromatids attached to the mitotic spindle are aligned at the center of the spindle [17]. These results demonstrated that JNKi-induced mitotic arrest was due to blockage at prometaphase as a result of the mitotic spindle checkpoint activation caused by mitotic spindle defects and failure of the chromosomes to assemble at the equatorial plate. These results also indicated that the microtubule network was the primary intracellular target for the anti-proliferative activity of JNKi.

3.3. Effect of APC on the JNKi-induced prometaphase-arrest, phosphorylation of Bcl-2, Mcl-1, and Bim, Bak activation, mitochondrial damage, and subsequent activation of caspase-3

To further examine the dependency of JNKi-induced apoptotic events on the prometaphase arrest of the cell cycle, we investigated the effect of APC, which blocks the cell cycle at the G_1/S border by inhibiting DNA polymerase α [15], on the JNKi-induced apoptotic events. When JT/Neo cells were treated with 0.75 μM APC for 20 h, 62.3% of the cells were in the G_1 phase; 26.2% in

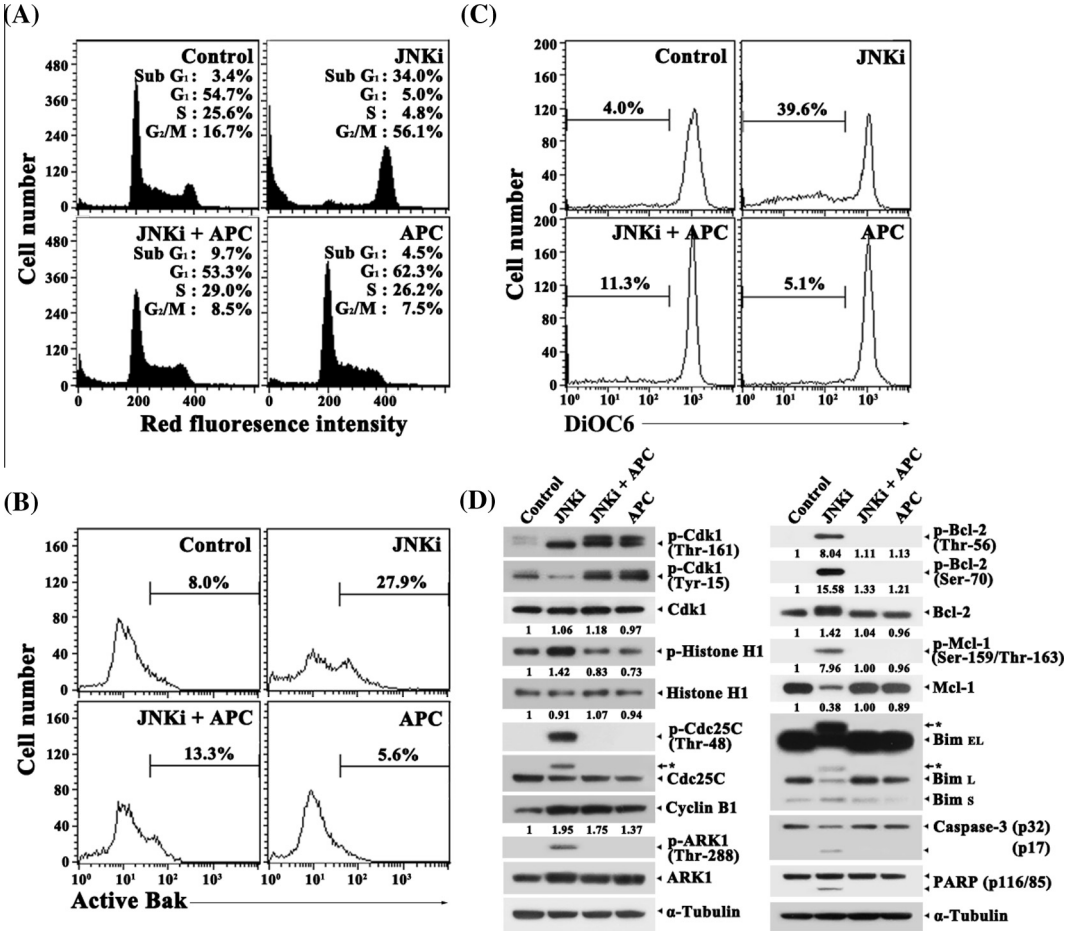

Fig. 3. Inhibitory effect of APC on the JNKi-induced G_2/M arrest (A), Bak activation (B), Δψm loss (C), and Western blot analysis of Cdk1 phosphorylation (Thr-161 and Tyr-15), cyclin B1 expression, histone H1 phosphorylation, Cdc25C phosphorylation (Thr-48), Bcl-2 phosphorylation (Thr-56 and Ser-70), Mcl-1 phosphorylation (Ser-159/Thr-163), Mcl-1 expression, reduction in the electrophoretic mobility of the Bim isoforms (BimEL and BimL), caspase-3 activation, PARP cleavage, and α-tubulin (D) in JT/Neo cells. The cells were treated with 50 nM JNKi, 50 nM JNKi and 0.75 μM APC, or 0.75 μM APC for 20 h. Cell cycle distribution, Bak activation, and loss of Δψm were analyzed as described in Section 2. Symbol: ←*, phosphorylated form of Cdc25C. A representative result is presented, and two additional experiments yielded similar results.

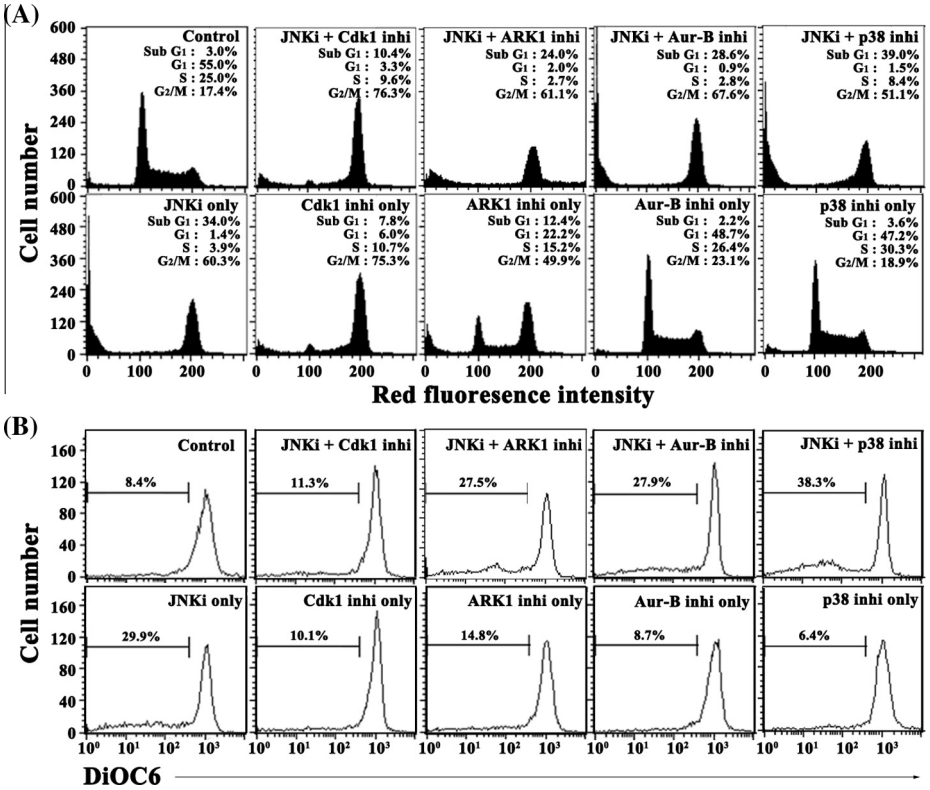

Fig. 4. Change in the cell cycle distribution (A) and $\Delta\psi$m (B) in JT/Neo cells after treatment with JNKi in the absence or presence of a Cdk1 inhibitor RO3306 (4 μM), an ARK inhibitor MLN8237 (12.5 nM), an Aurora B kinase inhibitor AZD1152-HQPA (12.5 nM) or a p38 MAPK inhibitor SB202190 (10 μM). The cells were preincubated with the individual inhibitors for 1 h and then treated with 50 nM JNKi for 20 h. Cell cycle distribution and $\Delta\psi$m loss were analyzed as described in Section 2. A representative result is presented, and two additional experiments yielded similar results.

the S phase, suggesting that the majority of the cells became arrested at the G_1/S border (Fig. 3A). Treatment of JT/Neo cells with 50 nM JNKi for 20 h caused 34.0% of the cells to be in the apoptotic sub-G_1 phase, along with 56.1% of the cells in the G_2/M phases. In the presence of APC, the JNKi-induced G_2/M arrest and the apoptotic sub-G_1 peak were almost completely abrogated, as were the JNKi-induced Bak activation and $\Delta\psi$m loss (Fig. 3B and C). These results indicated that JNKi failed to induce Bak activation, $\Delta\psi$m loss, and apoptosis when the majority of JT/Neo cells were forced to accumulate at the G_1/S border due to concomitant treatment with APC.

Western blot analysis also revealed that the JNKi-induced Bcl-2 phosphorylation at Thr-56 and Ser-70, Mcl-1 phosphorylation at Ser-159 and/or Thr-163, and Bim phosphorylation, activation of caspase-3, and PARP cleavage were not or were barely detected in the presence of APC (Fig. 3D). In addition, the JNKi-induced Cdk1 phosphorylation at Thr-161, Cdk1 dephosphorylation at Tyr-15, and Cdc25C phosphorylation at Thr-48, and histone H1 phosphorylation were not detected. These results demonstrated that the JNKi-induced apoptotic events, including Cdk1 activation, phosphorylation of Bcl-2, Mcl-1, and Bim, Bak activation, mitochondria-dependent activation of caspase-3, and cleavage of PARP were provoked as a consequence of mitotic prometaphase arrest.

3.4. Effect of Cdk1 inhibitor, ARK1 inhibitor, Aurora B kinase inhibitor, p38 MAPK inhibitor and pan-caspase inhibitor on the JNKi-induced apoptotic sub-G_1 peak and $\Delta\psi$m loss in JT/Neo cells

Previously, it has been shown that prolonged Cdk1 activation is crucial for prometaphase arrest-dependent apoptosis, which is provoked by microtubule-damaging drugs [17,21]. To examine the contribution of Cdk1, ARK1, and Aurora B kinase, and p38 MAPK to JNKi-induced apoptosis, we investigated the effect of a Cdk1 inhibitor (RO3306), an ARK1 inhibitor (MLN8237), an Aurora B kinase inhibitor (AZD1152-HQPA), and a p38 MAPK inhibitor (SB202190) on the JNKi-induced apoptotic sub-G_1 peak and $\Delta\psi$m loss in JT/Neo cells. Although the JNKi-induced sub-G_1 peak and $\Delta\psi$m loss reached 34.0% and 29.9%, respectively, these apoptotic events were significantly suppressed by RO3306, but not the other kinase inhibitors (Fig. 4A and B). Consequently, these results suggested that the induced phosphorylation of Bcl-2 family members Bcl-2, Mcl-1, and Bim in JT/Neo cells following exposure to JNKi, which led to mitochondrial damage-mediated apoptosis, was dictated by Cdk1 rather than ARK1, Aurora B kinase, or p38 MAPK. Since Bcl-2, Mcl-1, and Bim appeared to be reside predominantly on the mitochondrial outer membrane [21,24], it raised the possibility that the active Cdk1 might directly phosphorylate the mitochondrial proteins during 2-MeO-E_2-induced prometaphase arrest. It, however, remains unclear as to how Cdk1 phosphorylate concomitantly Bcl-2, Mcl-1, and Bim.

In conclusion, these results demonstrated that the JNKi-induced apoptotic signaling pathway, which led to apoptotic DNA fragmentation in Jurkat T cells, proceeded through prometaphase arrest of the cell cycle and Cdk1-mediated phosphorylation of Bcl-2 family members Bcl-2, Mcl-1, and Bim. These effects rendered the cells susceptible to the onset of Bak activation, leading to $\Delta\psi$m loss, and resultant caspase cascade activation. These results also provide an insight into the mechanism that underlies the pro-apoptotic role of Cdk1 during prometaphase arrest by a mitotic spindle-damaging action of JNKi-mediated inhibition of cellular JNK2 and JNK3.

Conflict of interest

All the authors report no conflicts of interest.

Acknowledgments

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