

Anisomycin induces apoptosis of glucocorticoid resistant acute lymphoblastic leukemia CEM-C1 cells via activation of mitogen-activated protein kinases p38 and JNK

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Glucocorticoids (GCs) resistance is frequently encountered in children with acute lymphoblastic leukemia (ALL), especially T-ALL, which usually results in failure of treatment. To find new agent to overcome GC resistance of ALL is an urgent problem. Here we investigated potential effect of anisomycin on GC-resistant T-ALL CEM-C1 cells and explored involved molecular mechanisms. Dramatic growth inhibition and apoptosis in GC-resistant CEM-C1 cells and GC-sensitive CEM-C7 cells induced by anisomycin were observed, which presented in a concentration- and time-dependent manner. Correspondingly, anisomycin induced cleaved caspase-3 and up-regulation of pro-apoptotic proteins (BimEL and Bad), meanwhile down-regulation of anti-apoptotic proteins (Mcl-1 and Bcl-2), both in a dose- and time-dependent manner in GC resistant CEM-C1 cells. Anisomycin also induced cell cycle arrest at G0/G1 phase in CEM-C1 cells through increasing expressions of p21 and p27, and attenuating the expression of cyclinA. The rapid up-regulation of phosphorylated mitogen-activated protein kinases (MAPKs) p38 and Jun N-terminal kinase (JNK) were observed after CEM-C1 cells were incubated with anisomycin. The activation of p38-MAPK and JNK could be blocked by respective inhibitors (SB203580 for p38-MAPK and SP600125 for JNK) accompanied with the inhibition of apoptosis and changes of apoptosis-associated proteins in CEM-C1 cells. These results suggested that anisomycin induced apoptosis of CEM-C1 cells via activation of p38-MAPK and JNK, and might be an attractive new agent for treatment of GC-resistant ALL.

Key words: anisomycin, acute lymphoblastic leukemia (ALL), glucocorticoid resistance, apoptosis, p38-Mitogen-Activated Protein Kinase (p38-MAPK), Jun N-terminal kinase (JNK)

Glucocorticoids (GCs) have been used to treat hematological malignancies for decades, and constituted a central role in the treatment of acute lymphoblastic leukemia (ALL)[1]. The sensitivity of leukemic cells to GCs is strongly relevant to therapeutic efficacy and prognosis in childhood ALL [2], and the resistance to GCs is the most important reason for treatment failure [3]. Clinically, GC resistance occurs in 10%~30% of childhood primary ALL, especially in T-ALL, and up to 70% in relapsed ALL. Therefore, aiming at GC resistance in ALL, to explore new therapeutic agents is an urgent clinical program. Previous studies have demonstrated that GCs induce apoptosis in lymphoid cells through activation of glucocorticoid receptor (GR) which was phosphorylated at serine 211 mediated by p38-MAPK[4], and the GC sensitivity in lymphoid cells can be enhanced by activation of p38-MAPK[5]. Hence, we supposed that the p38-MAPK agonists maybe possess the potential of

inducing apoptosis in GC-resistant ALL cells through activating p38-MAPK. Anisomycin, known as a protein synthesis inhibitor, is also an agonist of p38-MAPK[6], and has been used to induce apoptosis in some solid tumors[7-13]. Here, we reported for the first time that anisomycin induced dramatic apoptosis of GC-resistant human T-ALL CEM-C1 cells via activation of p38-MAPK and JNK.

Materials and methods

Cell lines and reagents. Human T-ALL cell lines of GC-sensitive CEM-C7-14 and GC-resistant CEM-C1-15 were kindly gifted by E.B.Thompson (University of Texas Medical Branch, Galveston, TX). We will refer to these cell lines as CEM-C7 and CEM-C1 in the following text. Dexamethasone (DEX, D1756), anisomycin (A9789), SB203580 (S8307) and

SP600125 (S5567) were all obtained from Sigma Aldrich and dissolved in ethanol or dimethyl sulfoxide (DMSO). MTT and Propidium iodide (PI) were purchased from Sigma. Annexin V-PI Kit was purchased from Keygen (Nanjing, China). Antibodies for p38, phosphorylated p38(p-p38), Jun N-terminal kinase 1 (JNK1), phosphorylated JNK (p-JNK), Bad, Bim, Bax, Bcl-2, Mcl-1, caspase-3 (cleaved at Asp175), cyclinA, cyclinD1 and the secondary antibodies of horseradish peroxidase (HRP)-conjugated sheep anti-rabbit antibody and HRP-conjugated sheep anti-mouse antibody were all obtained from Santa Cruz Biotech (USA). Antibodies for p21 and p27 were purchased from BD Bioscience (San Jose, CA, USA) and Cell Signaling Technology (Beverly, MA, USA), respectively. Anti-GAPDH antibody was obtained from Kangcheng Bio-Tech (Shanghai, China). ECL plus came from Amersham Biosciences(Inc., Piscataway, NJ).

Cell culture. Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2mM glutamine, 100U/ml penicillin/streptomycin and cultured at 37°C in a 5% CO₂ humidified atmosphere.

Detection of cell growth inhibition by MTT test. Cells in logarithmic growth phase were seeded in 96-well plate (2×10⁴ cells per well) with different final concentrations of anisomycin (0.1μmol/L, 1μmol/L and 10μmol/L) and incubated for various times. Then, 20μL MTT solution (5mg/mL) was added to each well and cells were incubated for additional 4h at 37°C and centrifuged at 1000 rpm for 10 min. The supernatant was gently removed from cell pellet and 150μl of DMSO (Sigma, USA) was added to each well. The plate was gently shaken for 10minutes and the absorbance at 570 nm was measured with an ELISA reader. Three separate experiments were conducted and each was performed in triplicate. The inhibition ratio of ALL cells was calculated based on the formula: Inhibition rate (%) = (1-absorbance of the experimental group/absorbance of the control group) ×100%.

Morphology observation of apoptosis by Wright's Giemsa staining. Cells in logarithmic growth phase were seeded in 24-well plate with the density of 2×10⁵/ml and incubated with 1μmol/L anisomycin for 12h. Then, cells were collected, washed, re-suspended and spin down (800rpm, 3min) onto glass slides. After staining with Wright's Giemsa, the morphology of cells was observed under light microscopy.

Measurement of apoptosis and cell cycle by flow cytometry. Cells in logarithmic growth phase were seeded in 6-well plate with the density of 2×10⁵/ml and incubated with different concentrations of anisomycin (0.1μmol/L, 1μmol/L and 10μmol/L) for 6h (for cell cycle analysis) or 12h (for detection of apoptosis), respectively. The cells were collected, washed and re-suspended at 1×10⁶ cells/ml in 500μl binding buffer containing 5μl of Annexin V-FITC stock solution and 10μl of PI. After incubated for 10min at room temperature in the dark, the apoptosis of cells was detected by flow cytometry. For cell cycle analysis, cells were fixed overnight in 70% ethanol at 4°C, washed, stained with 5μg/ml PI and analyzed by flow cytometry. For investigating the effect of inhibitors on

apoptosis (section 3.4), cells were preincubated with inhibitors of p38-MAPK and JNK respectively for 1 h, followed by incubation with anisomycin for 24h, then the apoptosis of cells was detected as described above.

Western blot analysis of proteins. CEM-C1 cells in logarithmic growth phase were treated with DEX or anisomycin for the indicated time points. Then, cells were harvested, washed and lysed in Laemmli sample buffer (Bio-Rad, Hercules, CA, USA). Protein concentration was determined by Coomassie brilliant blue. Samples were boiled for 5 min at 100°C and insoluble material was removed by centrifugation. 25μg-40μg proteins were separated on 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes (0.2μm, Millipore, São Paulo, SP, Brazil). The membranes were incubated with primary antibody at 4°C overnight. After washing 3 times with TBST, the membranes were incubated for 2h at room temperature with secondary HRP-conjugated sheep anti-rabbit antibody or HRP-conjugated sheep anti-mouse antibody diluted 1:3000 in TBST with 5% non-fat milk. Proteins were visualized using the ECL detection system (Amersham Biosciences, Oxford, United Kingdom). For investigating the effect of inhibitors (section 3.4), cells were preincubated with inhibitors of p38-MAPK and JNK respectively for 1 h prior to anisomycin treatment, and at the indicated time points, proteins were extracted and detected by western blotting as described above. The level of the GAPDH was used as a control of the amount of protein loaded into each lane and the quantification of the density of the bands on the blots was performed using Image J software. All experiments were carried out independently at least 3 times.

Statistical analysis. All experiments were performed in triplicate, and data were expressed as mean values ±SD. All statistical analyses were carried out with SPSS 17.0 software and multiple groups were compared with One-way ANOVA. Values were considered statistically significant with *p*-value < 0.05.

Results

Anisomycin induces growth inhibition on both GC-resistant CEM-C1 cells and GC-sensitive CEM-C7 cells. Both CEM-C1 and CEM-C7 cells belong to T-ALL cell lines, but they have absolutely opposite sensitivity to GCs. The previous studies by Thompsom et al. [14] and our group [15] have confirmed that CEM-C7 cells were very sensitive to DEX, while CEM-C1 cells were highly resistant to DEX. To test whether anisomycin induces growth inhibition on GC-resistant CEM-C1 cells, we first treated CEM-C1 cells with different concentrations of anisomycin for various times and used CEM-C7 cells as a control. As shown in Fig.1, just 0.1μmol/L anisomycin treated for 24h had a remarkable growth inhibition on CEM-C1 and CEM-C7 cells, and the inhibition rates were 46.68%±5.28% and 49.00%±7.57%, respectively. The high concentration of anisomycin (10μmol/L) manifested strong growth inhibition on both cells after only 6h incubation, and the inhibition rates were nearly same as that of 24h incubation with 0.1μmol/L

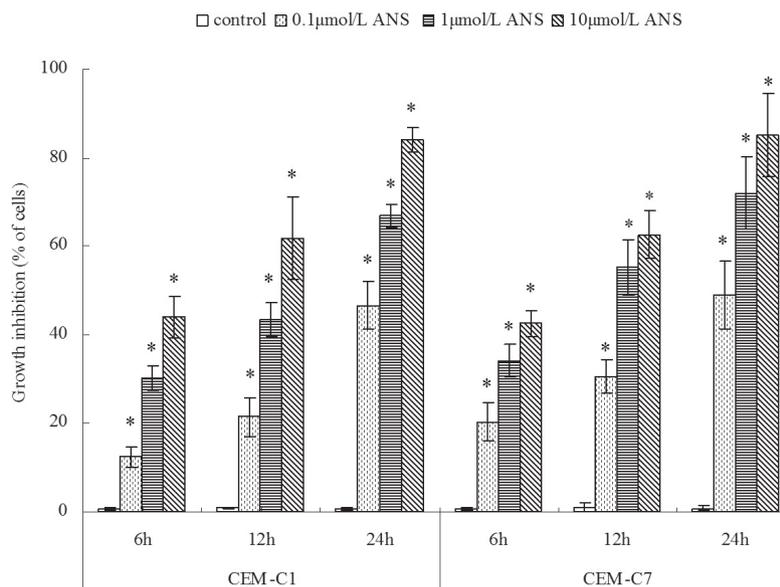


Figure 1. Growth inhibition of GC-resistant CEM-C1 cells and GC-sensitive CEM-C7 cells induced by anisomycin. Both CEM-C1 and CEM-C7 cells were incubated with different concentrations of anisomycin (ANS, 0.1 μmol/L, 1 μmol/L and 10 μmol/L) for 6h, 12h, 24h, respectively. The inhibition rate of cells was evaluated by MTT assay. Values represent the mean ± S.D. of three independent experiments. *p < 0.05 as compared with control group.

anisomycin. These results indicated that anisomycin had a powerful growth inhibition on both GC-resistant CEM-C1 cells and GC-sensitive CEM-C7 cells, and the effect presented in a concentration- and time-dependent manner.

Anisomycin induces apoptosis in both GC-resistant CEM-C1 cells and GC-sensitive CEM-C7 cells. As shown in Fig. 2A, after incubation with 0.1 μmol/L anisomycin for 12h, CEM-C1 cells displayed apoptotic bodies occasionally, while CEM-C1 cells showed typical feature of apoptosis, including cell shrinkage, apoptotic bodies and chromatin condensation to nuclear membrane after incubation with 1 μmol/L anisomycin for 12h, and when CEM-C1 cells were incubated with 10 μmol/L anisomycin for 12h, nuclear fragmentation and cell debris appeared clearly besides apoptotic bodies. CEM-C7 cells also had apoptosis in a concentration-dependent manner and mainly manifested as apoptotic bodies formation, cell shrinkage and cell debris. Meanwhile, we treated CEM-C1 and CEM-C7 cells with different concentrations of anisomycin for 12h to detect apoptosis by Annexin V-FITC/PI staining and flow cytometry. As shown in Fig. 2B, The apoptosis induced by anisomycin presented in a concentration-dependent manner in both CEM-C1 and CEM-C7 cells. These data indicated that anisomycin could induce apoptosis not only in GC-sensitive CEM-C7 cells but also in GC-resistant CEM-C1 cells.

To further test pathogenesis of anisomycin-induced apoptosis in CEM-C1 cells, we selectively detected expression changes of Bcl-2 family proteins which were thought to be associated with GC resistance [16-18]. As shown in Fig. 3A, the expressions of pro-apoptotic proteins BimEL and Bad in CEM-C1 cells were up-regulated while anti-apoptotic proteins Bcl-2 and Mcl-1 were down-regulated, both presenting in

a concentration-dependent manner. Cleaved caspase-3 also increased. In addition, different changes of Bcl-2 family proteins were also observed when CEM-C1 cells were incubated with 1 μmol/L anisomycin for different times. As shown in Fig. 3B the expression of pro-apoptotic protein BimEL was increased as early as 30min after cells were incubated with anisomycin, and continued high expression up to 48h. The expression of Bad began increasing 1h after CEM-C1 cells were incubated with 1 μmol/L anisomycin, and kept high expression until 24h and then decreased. There was no obvious change in Bax. In contrast to pro-apoptotic proteins, the expression of anti-apoptotic proteins Mcl-1 markedly decreased 30min after CEM-C1 cells were incubated with 1 μmol/L anisomycin and kept low expression until 48h, meanwhile Bcl-2 began decreasing obviously at 6h, kept continuous decreasing and finally disappeared at 48h. Activation of caspase-3 (cleaved into p17 and p12 fragments) appeared 3h after CEM-C1 cells were treated with 1 μmol/L anisomycin, and kept significantly increased till 48h. These results suggested that anisomycin induced apoptosis in CEM-C1 cells through mitochondrial apoptotic pathway.

Anisomycin induces cell cycle arrest in both GC-resistant CEM-C1 cells and GC-sensitive CEM-C7 cells. Cell cycle regulation is an important process in cells undergoing apoptosis. To test whether anisomycin induced cell cycle arrest in GC-resistant CEM-C1 cells, different concentrations of anisomycin (0.1 μmol/L, 1 μmol/L and 10 μmol/L) were used to treat CEM-C1 and CEM-C7 cells (as a control) for 6h and cell cycle arrest was detected by PI staining and flow cytometry. As shown in Fig. 4, anisomycin induced a significant increased proportion of G0/G1 phase, and a simultaneously decreased

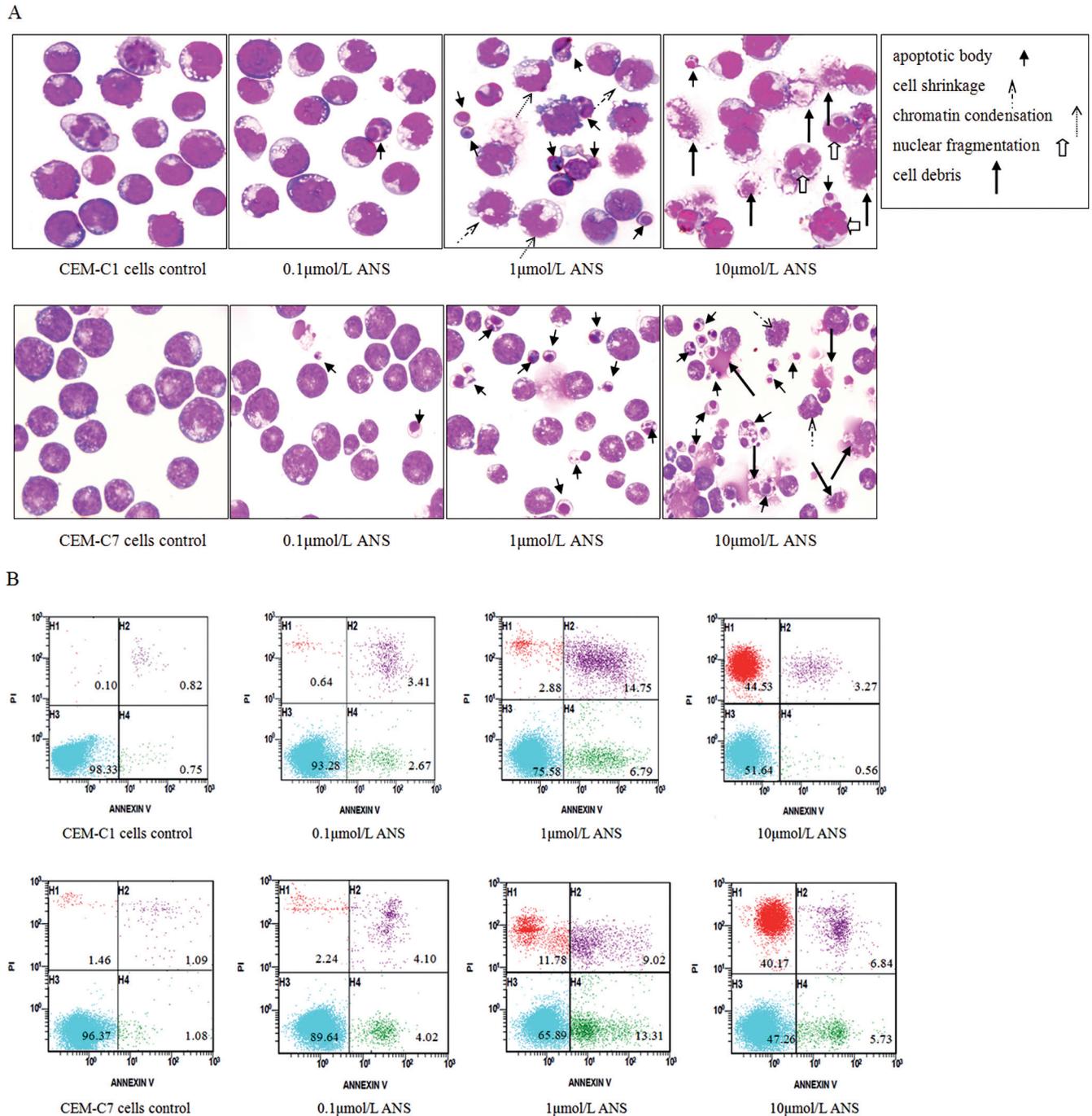


Figure 2. Apoptosis of GC-resistant CEM-C1 cells and GC-sensitive CEM-C7 cells induced by anisomycin. CEM-C1 and CEM-C7 cells were incubated with different concentrations of ANS (anisomycin, 0.1 μmol/L, 1 μmol/L and 10 μmol/L) for 12h, respectively. (A) The morphological changes of cells were examined by Wright's Giemsa staining and then observed under microscope at a magnification of 10×40. (B) The percentage of apoptotic cells was determined by Annexin V-FITC/PI staining and flow cytometry. Percentages of each gated fraction (%) have been marked on the pictures.

proportion of S and G2/M phase in both cell lines suggesting that anisomycin arrested both GC-sensitive CEM-C7 cells and GC-resistant CEM-C1 cells at G0/G1 phase in a concentration-dependent manner.

To explore the molecular mechanisms of anisomycin-induced cell cycle arrest, we investigated the expressions of cell cycle regulatory proteins in CEM-C1 cells. As shown in Fig.5A, anisomycin induced up-regulation of cyclinD1, p21 and p27

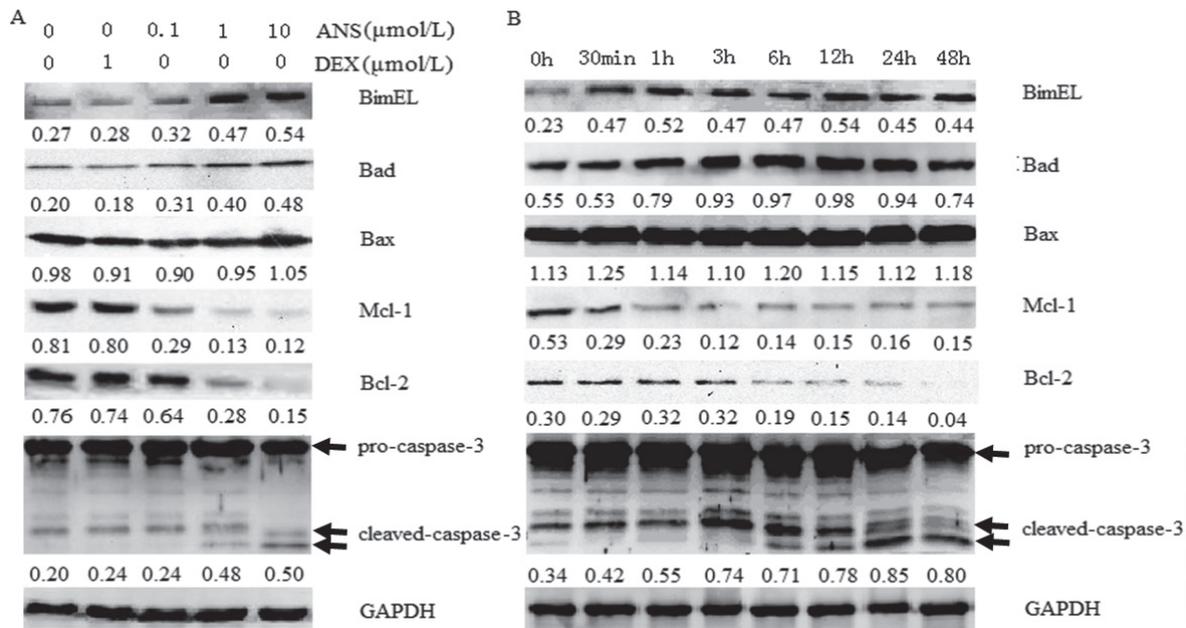


Figure 3. Expressions of Bcl-2 family proteins and caspase-3 in GC-resistant CEM-C1 cells induced by anisomycin. CEM-C1 cells were incubated with (A) the indicated concentrations of DEX or ANS (anisomycin) for 6h or (B) 1 μ mol/L anisomycin for the indicated times, and then cells were collected, lysed and extracts were subjected to western blot analysis. BimEL, Bad, Bax, Mcl-1, Bcl-2 and caspase-3 were detected by using specific antibodies against these proteins. The data of densitometry which standardized by GAPDH are presented below the band.

while down-regulation of cyclinA in a concentration-dependent manner. When CEM-C1 cells were treated with 1 μ mol/L anisomycin for different times (Fig.5B), the expression of cyclin A decreased obviously after 6h and kept decline up to 48h, while the expression of cyclin D1 increased after 30min and kept elevated up to 48h. P27 and p21 increased gradually after 30min (p27) and 3h (p21) respectively, continued increasing up to 12h (p27) and 24h (p21), and then both decreased. These results indicated that anisomycin arrested CEM-C1 cells at G0/G1 phase through up-regulating p27 and p21 and down-regulating cyclin A in a concentration- and time-dependent manner.

Anisomycin induces p38-MAPK and JNK activation in GC-resistant CEM-C1 cells. As shown in Fig.6, the expression of phosphorylated p38-MAPK (p-p38-MAPK) in CEM-C1 cells treated with anisomycin began increasing after 30min incubation, arrived at peak after 1h, and then decreased after 3h, presenting in a time-dependent manner. Unlike p-p38-MAPK, phosphorylated JNK (p-JNK) was rapidly activated, arrived at peak just after 30min and almost disappeared after 3h. To test whether the two kinases have a specific role in anisomycin-induced apoptosis, the respective inhibitors (SB203580 for p38-MAPK, SP600125 for JNK) were used to treat CEM-C1 cells for 1 h prior to anisomycin treatment. As shown in Fig.7, the activation of p38-MAPK and JNK in CEM-C1 cells induced by anisomycin could be blocked by respective specific inhibitors, which was accompanied with changes of cell apoptosis and expressions

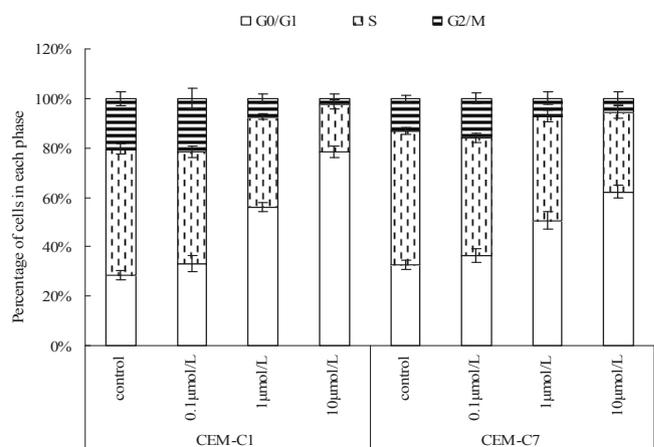


Figure 4. Cell cycle arrest of GC-resistant CEM-C1 cells and GC-sensitive CEM-C7 cells induced by anisomycin. CEM-C1 and CEM-C7 cells were incubated with different concentrations of anisomycin (0.1 μ mol/L, 1 μ mol/L and 10 μ mol/L) for 6h, respectively. Then the percentage of cells at each stage of the cell cycle was detected by PI staining and flow cytometry. Values represent the mean \pm S.D. of three independent experiments.

of Bcl-2, Mcl-1 and BimEL, indicating anisomycin-induced apoptosis in CEM-C1 cells might be through p38-MAPK and JNK signal pathways.

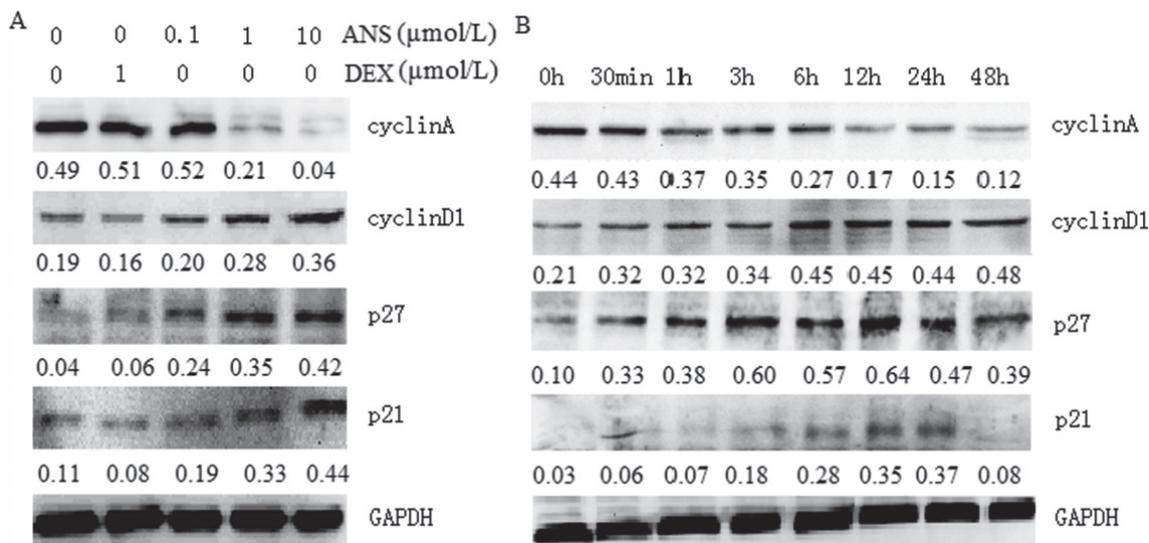


Figure 5. Expressions of cell cycle regulatory proteins in GC-resistant CEM-C1 cells induced by anisomycin. CEM-C1 cells were incubated with (A) the indicated concentrations of DEX or ANS (anisomycin) for 6h or (B) 1μmol/L anisomycin for the indicated times, and then cells were collected, lysed and extracts were subjected to western blot analysis. CyclinA, cyclinD1, p27 and p21 were detected by using specific antibodies against these proteins. The data of densitometry which standardized by GAPDH are presented below the band.

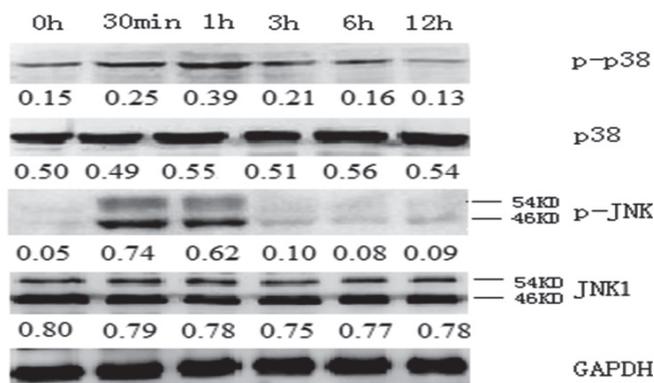


Figure 6. Expressions of phosphorylated p38 and JNK in GC-resistant CEM-C1 cells induced by anisomycin. CEM-C1 cells were incubated with 1μmol/L anisomycin for indicated times, and then cells were collected, lysed and extracts were subjected to western blot analysis. P-p38 (Thr-180/Tyr-182), p38, p-JNK (Thr-183/Tyr-185), and JNK1 were detected by using specific antibodies against these proteins. The data of densitometry which standardized by GAPDH are presented below the band.

Discussion

Anisomycin induces apoptosis of CEM-C1 cells through regulating the expression of pro- and anti-apoptotic proteins. The apoptosis of most tumor cells induced by chemotherapeutic drugs proceeds through the mitochondria pathway, and the Bcl-2 family proteins play a pivotal role. The pro-apoptotic Bim is of important role in GC resistance of ALL. Previous studies have

demonstrated that the absence of Bim up-regulation after treated with DEX appears to be one of the most important mechanisms of GC resistance in ALL cells [15,18-19]. Bim mediates apoptosis by binding to anti-apoptotic members of the Bcl-2 family (e.g., Bcl-2, Mcl-1 and Bcl-xL) [20] or by activating pro-apoptotic members Bax and Bak[21]. Bad, another important pro-apoptotic factor, is mainly distributed in cytoplasm and promotes cells to apoptosis by forming hetero-dimer with anti-apoptotic Bcl-2 and Bcl-XL[21]. Otherwise, two anti-apoptotic proteins in Bcl-2 family are associated with GC resistance of ALL. Mcl-1, usually over-expressed in a variety of human cancers including GC-resistant MLL rearranged infant ALL[16,22], promotes cells survive by forming hetero-dimer with pro-apoptotic proteins (such as Bax, Bak or Bim) to prevent the releasing of cytochrome c[21]. Bcl-2 is also over-expressed in many kinds of cancer cells and tightly related to drug resistance including GC resistance [23,24]. The increase of Bcl-2/Bax ratio is related to chemotherapeutic drug resistance in B cell chronic lymphocytic leukemia[25] and staurosporine can successfully reverse the GC resistance of T lymphoma cells by down-regulating the expression of Bcl-2[24].

In present study, pro-apoptotic Bim and Bad were remarkably up-regulated whereas anti-apoptotic Mcl-1 and Bcl-2 were significantly down-regulated in anisomycin-induced apoptosis of GC-resistant CEM-C1 cells, both of which presented in a concentration- and time-dependent manner (Fig.3). Furthermore, caspase-3, the executioner of both death receptor and mitochondrial pathway of apoptosis, was also activated in anisomycin-triggered apoptosis of CEM-C1 cells (Fig.3). These results suggested that anisomycin induced apoptosis in GC-resistant CEM-C1 cells mainly

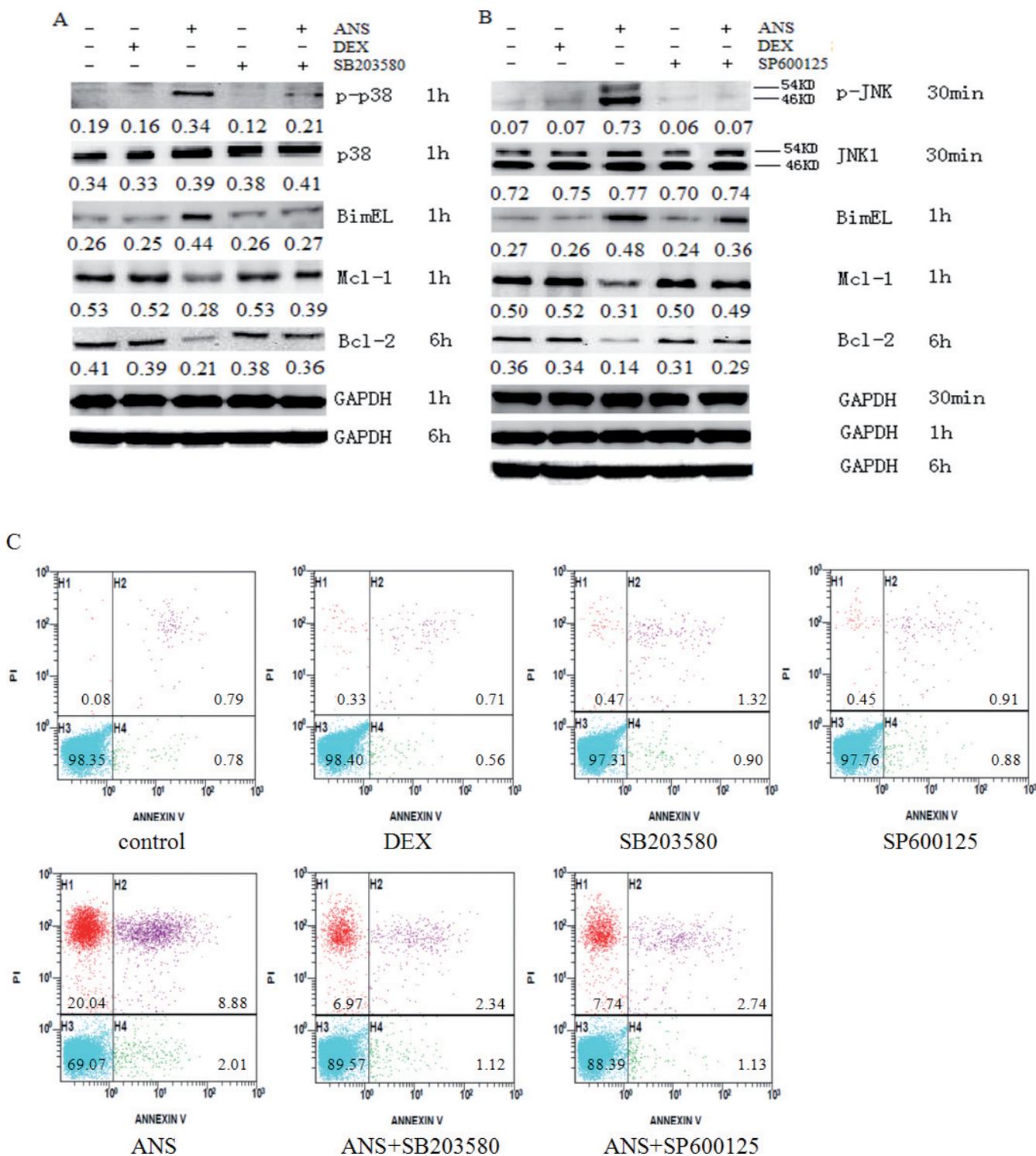


Figure 7. The anisomycin-induced activation of p38 and JNK in GC-resistant CEM-C1 cells could be blocked by inhibitors of p38(SB203580) and JNK(SP600125). CEM-C1 cells were incubated with no drugs or $1\mu\text{mol/L}$ DEX or $1\mu\text{mol/L}$ ANS (anisomycin) or inhibitors ($50\mu\text{mol/L}$ SB203580 or $50\mu\text{mol/L}$ SP600125) or combination of $1\mu\text{mol/L}$ anisomycin and inhibitors. In the co-treatment groups, cells were preincubated with inhibitors for 1 h prior to anisomycin treatment. (A) and (B) at the indicated time points, cells were collected, lysed and extracts were subjected to western blot analysis. P-p38 (Thr-180/Tyr-182), p38, p-JNK(Thr-183/Tyr-185), JNK1, BimEL, Mcl-1 and Bcl-2 were detected by using specific antibodies against these proteins. The data of densitometry which standardized by GAPDH are presented below the band. (C) after 24h incubation, the percentage of apoptotic cells was determined by Annexin V-FITC/PI staining and flow cytometry. Percentages of each gated fraction (%) have been marked on the pictures.

through mitochondria pathway and by regulating capital Bcl-2 family proteins which are tightly associated with GC resistance.

Anisomycin induces cell cycle arrest in CEM-C1 cells through modulating the expression of cell cycle-associated proteins. Proper regulation of cell cycle plays a fundamental role in organisms, as it impacts on cellular division, differentiation and death. In this intricate process, cyclins, together with cyclin-dependent kinases (CDKs) govern cell cycle progression in eukaryotic cells. Progression through the phases of cell cycle relies on cyclin/CDKs complexes that are regulated by multiple cyclin-dependent kinase inhibitors (CKI). CyclinD1 and cyclinA are two important positive agents of cell cycle. CyclinD1 promotes cells to pass G1/S checkpoint and enter S phase, and cyclinA is required for DNA replication in S phase. p21 (Cip1) and p27(Kip1) are two important CKIs which negatively regulate cell cycle. p21 interacts with and inhibits various cyclin/CDKs complexes, specifically blocks the initiation of DNA replication and strongly inhibits cyclinA[26]. p27, as a potent tumor-suppressor, can block the phosphorylation of CDKs or directly inhibits the activity of cyclin/CDKs[27]. Previous studies have indicated that cyclinA and cyclinD1 have higher expression while p27(Kip1) has lower expression in childhood and adult leukemia which may contribute to the pathogenesis and the progress of the leukemia[28-30]. In addition, reports also confirmed that the increase of p21 and p27 can inhibit growth of leukemia cells [31].

Although anisomycin can arrest cells at G0/G1 phase [13], the reports about anisomycin regulating cell cycle-related proteins are rare. In present study, anisomycin induced the up-regulation of p21 and p27, and down-regulation of cyclinA in GC-resistant CEM-C1 cells, but it also induced an unexpected up-regulation of cyclinD1. Recent study found that, in addition to promoting cells to pass G1/S checkpoint and enter S phase, cyclinD1 can directly binds RAD51, a recombinase that drives the homologous recombination process in DNA repair induced by radiation, and reduction of cyclinD1 levels in human cancer cells impairs recruitment of RAD51 to damaged DNA, impedes the homologous recombination-mediated DNA repair, and increases sensitivity of cells to radiation in vitro and in vivo[32]. We supposed that anisomycin-induced apoptosis of CEM-C1 cells was accompanied with DNA damage which might induce the up-regulation of cyclinD1 for DNA repair, but meanwhile decreased cyclinA impeded DNA replication, and additionally up-regulation of p21 and p27 inhibited the activity of cyclinD1. Therefore the integration of anisomycin was to impeded CEM-C1 cells to enter S phase and arrested cells at G1 phase, which was coincident with the results detected by flow cytometry (Fig.4).

JNK and p38-MAPK are involved in anisomycin-induced apoptosis of GC-resistant CEM-C1 cells. Apoptosis of cells is an extremely coordinated phenomenon which involves a series of signaling molecules like stress kinases, caspases and Bcl-2

family proteins[33]. It has been reported that the JNK and p38-MAPK, as two members of MAPK family, are associated with apoptosis of ALL.

The JNK, activated by MAPK cascade, specifically phosphorylates and regulates the activity of transcription factors, nontranscription factors, and also has a central role in regulating many cellular activities from cell cycle progression to apoptosis[34]. JNK has been clouded by controversies because of its role both as an anti-apoptotic and a pro-apoptotic mediator[33]. Treatment of T-ALL cells with JNK inhibitors led to cell cycle arrest and apoptosis, and increased sensitivity to Fas-mediated apoptosis[34]. Activation of the JNK pathway promoted phosphorylation and degradation of BimEL in T-ALL Sup-T1 cells and conferred resistance of Sup-T1 cells to etoposide-induced apoptosis[35]. However, the apoptosis of P-gp over-expressed T-ALL cells induced by perifosine was in part dependent on the Fas/FasL interaction and JNK activation, and the effect of perifosine-induced down-regulation of P-gp required JNK activity [36]. There were also reports about activation of JNK in anisomycin-induced apoptosis of solid tumor[12]. These studies indicate that JNK has promoting or inhibiting apoptotic functions, depending on cell type, nature of the death stimulus, duration of its activation and the activity of other signaling pathways[37].

The p38-MAPK, activated by phosphorylation at tyrosine or serine/threonine sites, participates in the process of apoptosis [38]. Studies have demonstrated that p38-MAPK plays an important role in GC-induced up-regulation of Bim and apoptosis in ALL [4,15,39]. Activated p38-MAPK phosphorylates glucocorticoid receptor at serine 211 and triggers cascade of GC-induced apoptosis of ALL[4].

In present study, rapid activation of JNK and p38-MAPK in anisomycin-treated GC-resistant CEM-C1 cells were observed, followed by down-regulation of Mcl-1 and Bcl-2, and up-regulation of BimEL, all of three are important anti- or pro-apoptotic proteins associated with GC resistance in ALL. SP600125 and SB203580, the respective specific inhibitors of JNK and p38-MAPK, could significantly inhibit anisomycin-induced activation of JNK and p38-MAPK, also inhibited the anisomycin-induced apoptosis, down-regulation of Mcl-1 and Bcl-2 and up-regulation of Bim, suggesting that JNK and p38-MAPK signaling pathways are critical in anisomycin-induced apoptosis of CEM-C1 cells.

In conclusion, our results strongly suggest that anisomycin inhibits GC-resistant CEM-C1 cells growth, induces cell cycle arrest at the G0/G1 phase, and triggers CEM-C1 cells to apoptosis mainly by p38-MAPK and JNK signaling and mitochondrial apoptotic pathway. These results indicate that anisomycin might be a potential drug for treating GC-resistant ALL.

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