Methyl jasmonate downregulates expression of proliferating cell nuclear antigen and induces apoptosis in human neuroblastoma cell lines

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Recent evidence indicates that methyl jasmonate, a plant stress hormone, exhibits anticancer activity on human cancer cells. Whether methyl jasmonate could inhibit the growth of human neuroblastoma cells still, however, remains largely unknown. In this study, administration of methyl jasmonate to cultured neuroblastoma cell lines, SK-N-SH and BE(2)-C, resulted in a decrease of cell viability in a dose-dependent and time-dependent manner as demonstrated by MTT colorimetry and colony formation assay. The results from RT-PCR indicated that the expression of proliferating cell nuclear antigen, but not of cyclin D1, was downregulated by methyl jasmonate. Accordingly, the cell cycle of methyl jasmonate-treated neuroblastoma cells was arrested at the G_0/G_1 phase. Moreover, incubation of SK-N-SH and BE(2)-C cells with methyl jasmonate resulted in characteristic changes of apoptosis, as demonstrated by acridine orange–ethidium bromide (AO/EB) staining, Hoechst 33258 staining and flow cytometry. Moreover, methyl jasmonate decreased the expression of the X-linked inhibitor of apoptosis protein and survivin, critical members of the inhibitors of apoptosis protein family, in neuroblastoma cells. These findings indicate that methyl jasmonate suppresses the growth of cultured human neuroblastoma cells associated with downregulation of proliferating cell nuclear antigen, and induces apoptosis accompanied by downregulation of the X-linked inhibitor of apoptosis protein and survivin, which lays the groundwork for further investigation into the mechanisms of methyl jasmonate-mediated anticancer activities. *Anti-Cancer Drugs* **19:573–581 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Plant-derived compounds have been an important source of clinically useful anticancer agents, such as vinblastine, vincristine, topotecan, irinotecan, etoposide, and paclitaxel [1]. It has been evaluated that over 60% of the anticancer agents in use are derived from natural sources, such as plants, marine organisms, and microorganisms [2]. From a wider perspective, plant-based compounds continue to play an essential role in the primary health care of 80% of the world's population [2]. Out of about 250 000 species of plants, 1000 possess significant anticancer action [3].

Plant hormones are a group of naturally occurring organic substances that influence physiological processes of plants, such as growth, differentiation, and development, at low concentrations [4]. Previous evidence indicated that salicylate suppressed the proliferation of lymphoblastic leukemia, prostate cancer, and breast cancer [5,6]. At a plasma-attainable and nontoxic level, aspirin suppressed the proliferation of metastatic murine melanoma cells and human melanoma cells [7]. Additionally, administration of plasma-attainable concentrations of aspirin for 3 days resulted in reduced cellular proliferation by up to 35–55% in human prostate cancer cell lines [8]. Abscisic acid, another plant stress hormone, has also been reported to induce death in mouse leukemia cells [9]. Jasmonates, the major constituents in the etheric oil of jasmine, are potent lipid regulators that mediate responses to mechanical and infectious stresses throughout the plant kingdom [10]. It has been demonstrated that leaf tissues exhibit a fast increase in jasmonate levels in response to various stimuli and stresses, for example, wounding, herbivory, and infection. Jasmonates regulate plant gene expression including defense-related genes [10]. Interestingly, jasmonate perception in insects may allow the latter to defend themselves against jasmonate-induced toxicity, suggesting that jasmonate signaling pathways may be conserved between the plant and animal kingdoms [10].

In recent years, several groups have reported that members of the plant stress hormone family of jasmonates, and some of their synthetic derivatives, exhibited

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anticancer activity *in vitro* and *in vivo* [11]. Fingrut and Flescher [5] reported that jasmonates induced suppression of cellular proliferation and death in various human and mouse cancer cell lines, including breast cancer, prostate cancer, melanoma, lymphoblastic leukemia, and lymphoma cells. Jasmonates were also found to inhibit the proliferation and induce death of various other cancer cells including lung cancer and myeloid leukemia cells [12,13]. Up to now, three mechanisms of action have been proposed to explain the anticancer activities of jasmonates [14], including induction of severe ATP depletion in cancer cells via mitochondrial perturbation, induction of redifferentiation in human myeloid leukemia cells via mitogen-activated protein kinase activity, induction of reactive oxygen species-mediated apoptosis in lung carcinoma cells via generation of hydrogen peroxide and proapoptotic proteins of the Bcl-2 family. However, it still remains largely unknown whether jasmonates exert effects on the proliferation and apoptosis of human neuroblastoma cell lines. In this study, we demonstrated that methyl jasmonate suppressed the growth of cultured neuroblastoma cells associated with downregulation of proliferating cell nuclear antigen (PCNA), and induced apoptosis involving modulation of expression of X-linked inhibitor of apoptosis protein (XIAP) and survivin, critical members of the inhibitor of apoptosis protein (IAP) family.

Materials and methods

Cell culture and methyl jasmonate treatment

Human neuroblastoma cell lines, SK-N-SH and BE(2)-C, were purchased from the American Type Culture Collection (ATCC) and grown in minimal essential medium (Life Technologies, Inc., Gaithersburg, Maryland, USA) and supplemented with 10% fetal bovine serum (Life Technologies, Inc.), penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2. The methyl jasmonate (Sigma, St Louis, Missouri, USA) was prepared into stock solutions at a concentration of 1 mol/l in anhydrous dimethyl sulfoxide (DMSO; Sigma) and stored at −20°C. Confluent monolayers of cells were incublated with different concentrations of methyl jasmonate, cisplatin (Sigma) as positive control, or DMSO as indicated.

Measurement of cell viability

Cell viability was monitored by the 2-(4, 5-dimethyltriazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma) colorimetric assay [15]. Briefly, 20 μl of MTT (5 mg/ml) was added to each well. After 4 h of incubation at 37°C, the cell supernatants were discarded, MTT crystals were dissolved with DMSO and the absorbance measured at 570 nm. Percent viability was defined as the relative absorbance of treated versus untreated control cells. The 50% inhibitory concentration of 24 h exposure, defined as the drug concentration resulting in 50% reduction of cell viability compared with untreated control, was determined by Bliss's software (Bliss Co, California, USA). All experiments were carried out with 6–8 wells per experiment and repeated at least three times.

Colony formation assay

The cells were seeded at a density of 500/ml on 6-well plates [16]. After an overnight incubation to allow cell attachment, cells were incubated with methyl jasmonate, cisplatin, or DMSO as indicated. After incubation for 24 h, the medium was replaced with fresh medium containing 10% fetal bovine serum. Colonies were allowed to grow for 10–14 days. The medium was discarded and each well was washed carefully twice with phosphate buffered saline (PBS). The cells were fixed in methanol for 15 min and then stained with crystal violet for 20 min. Finally, positive colony formation (more than 50 cells/colony) was counted. The survival fraction for cells was expressed as the ratio of plating efficiency of treated cells to that of untreated control cells.

Cell cycle assay

According to the literature [17], cell cycles in untreated, DMSO-treated, methyl jasmonate-treated, or cisplatin-treated cells were examined by flow cytometry. Briefly, 2×10^5 of cells were collected, washed twice with 0.01 mol/l PBS and fixed in 70% ethanol overnight at 4°C. Then, cells were washed once with PBS, digested by 200 μl of RNase (1 mg/ml) at 37°C for 30 min, and stained with 800 μl of propidium iodide (PI, 50 μg/ml, Sigma) at room temperature for 30 min. The DNA histograms were generated with a flow cytometer (Becton Dickson Co., San Jose, California, USA), using the CELLQEST software (Becton Dickson).

Cellular morphological observation

To observe the changes in cellular morphology, the AO (Sigma) and EB (Sigma) staining method was performed [18]. Briefly, cells were harvested with 0.125% trypsin and 0.01% EDTA, resuspended in 95 μl of minimal essential medium, and incubated with 5 μl of AO/EB staining solution (100 mg/l PBS of each dye) at room temperature for 15 min. Cells were examined using fluorescence microscopy and photographed (Olympus, Tokyo, Japan). Viable cells were colored green with intact nuclei. Nonviable cells had bright orange chromatin. Apoptosis was demonstrated by the appearance of cell shrinkage with condensation and fragmentation of the nuclei. Necrotic cells appeared orange with a normal nuclear structure. The numbers of normal nuclei (VN), viable cells with apoptotic nuclei (VA), nonviable cells with apoptotic nuclei (NVA), and nonviable cells with normal nuclei (NVN) cells were determined by counting five randomly selected high-power (× 200) fields. Apoptosis rates were calculated as (%) = (VA + NVA)/(VN + NVN + VA + NVA) × 100%.

Morphological evidence of apoptosis was further obtained using the Hoechst 33258 staining method [19]. Briefly, cells were fixed with 4% formaldehyde in PBS solution at 4°C for 10 min. After three washes with PBS, the cells were stained with 10 mg/l of Hoechst 33258 in PBS solution at 4°C in the dark for 10 min, and morphologic changes including cell shrinkage and nuclear condensation were observed under a fluorescence microscope (Olympus AX80, Olympus).

Detection of apoptosis rates

Apoptotic ratios of cells were determinated by annexin V-FITC (BD Pharmingen, San Diego, California, USA) and PI staining flow cytometry [20]. Briefly, cells from the above groups were collected, washed twice with cold PBS, resuspended with 100 µl of binding buffer (10 mmol/l HEPES, 140 mmol/l NaCl, 2.5 mmol/l $CaCl_2$, pH 7.4) into 2–5 $\times$ 10^5 cells/ml density, and incubated with annexin V-FITC at room temperature for 10 min. After washing with binding buffer, the cells were resuspended with 400 µl of binding buffer containing 10 µl of PI (20 µg/ml), and incubated on ice for 15 min. Apoptosis was analyzed by a flow cytometer (Becton Dickson Co.) at a wavelength of 488 nm. This method can be used to distinguish between living cells (annexin V^-/PI^-), early apoptotic/primary apoptotic cells (annexin V^+/PI^-), late apoptotic/secondary necrotic cells (annexin V^+/PI^+), and necrotic cells (annexin V^-/PI^+) [20].

RT-PCR for PCNA, cyclin D1, X-linked inhibitor of apoptosis protein and survivin

Total RNA was isolated with RNeasy Mini Kit (Qiagen Inc., Valencia, California, USA). The reverse transcription reactions were conducted with Transcriptor First Strand cDNA Synthesis Kit (Roche, Indianapolis, Indiana, USA). The PCR primers were designed by Premier Primer 5.0 software as the following: for human PCNA 5′-AAAC TAGCTAGACTTTCCTC-3′ and 5′-TCACGCCCATG GCCAGGTTG-3′ amplifying a 274-bp fragment; for human cyclin D1 5′-TGCCGTCCATGCGGAAGAT-3′ and 5′-CACAGAGGGCAACGAAGGT-3′ amplifying a 411-bp fragment; for human XIAP 5′-GAACCTTGTGA TCGTGCCT-3′ and 5′-AGGGTCTTCACTGGGCTTC-3′ amplifying a 318-bp fragment; for human survivin 5′-CAAGGACCACCGCATCTCTA-3′ and 5′-TTCTTCG CAGTTTCCTCAA-3′ amplifying a 348-bp fragment; for human α-tubulin 5′-CTCATCACAGGCAAGGAAGAT-3′ and 5′-TTAAGGTAAGTGTAGGTTGGG-3′ amplifying a 410-bp fragment. The ratios between the amplified DNA fragments and α-tubulin for each sample RNA were quantified by Phoretix 1D software (Phoretix International Ltd., Newcastle upon Tyne, UK).

Statistical analysis

Unless otherwise stated, all data were shown as mean ± standard error of the mean. Statistical significance ($P < 0.05$) was determined by *t*-test or analysis of variance followed by assessment of differences using SPSS 12.0 software (SPSS Inc., Chicago, Illinois).

Results

Methyl jasmonate suppressed the growth of neuroblastoma cells

Earlier studies have indicated that jasmonates exerted extensive anticancer activities on cancer cells [5,12,13]. To explore the effects of methyl jasmonate on neuroblastoma cells, we first observed the cell viability changes in cultured SK-N-SH and BE(2)-C cell lines. As shown in Fig. 1a and b, the MTT colorimetric assay indicated that methyl jasmonate and cisplatin, but not DMSO (the dissolvent of methyl jasmonate), inhibited cell viability of SK-N-SH and BE(2)-C cells in a dose-dependent and time-dependent manner. The 50% inhibitory concentrations of methyl jasmonate on SK-N-SH and BE(2)-C cells were 1.39 and 1.35 mmol/l, respectively, which were higher than those of cisplatin (0.057 and 0.2 mmol/l, respectively). Colony formation assay further demonstrated that administration of methyl jasmonate and cisplatin, but not DMSO, resulted in decreased cell proliferation of SK-N-SH and BE(2)-C cells (Fig. 1c and d). These findings indicated that the methyl jasmonate suppressed the in-vitro growth of cultured neuroblastoma cell lines.

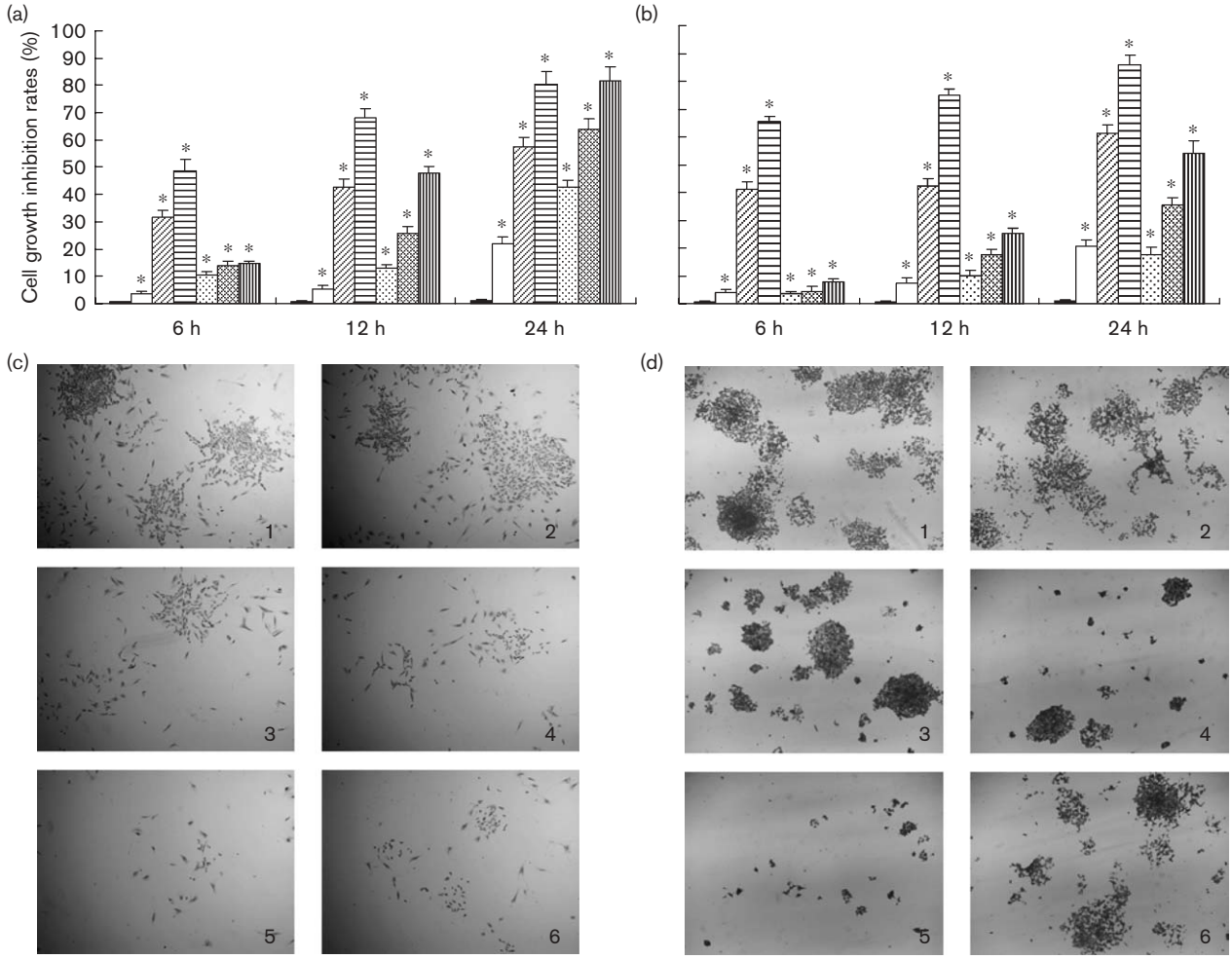
Methyl jasmonate downregulated PCNA, but not cyclin D1, in neuroblastoma cells

PCNA is an intranuclear polypeptide maximally synthesized during the S phase of cell cycle and participates in cell proliferation [21]. As the above evidence indicated that methyl jasmonate suppressed cell viability of neuroblastoma cells, we hypothesized that methyl jasmonate might modulate PCNA expression in these cells. To meet this end, SK-N-SH and BE(2)-C cells were treated with various concentrations of methyl jasmonate for 24 h. As shown in Fig. 2a and b, RT-PCR demonstrated that methyl jasmonate, but not DMSO or cisplatin, decreased PCNA mRNA in SK-N-SH and BE(2)-C cells in a dose-dependent manner. Administration of methyl jasmonate, however, resulted in no changes in cyclin D1 mRNA levels. The cell cycle assay showed that methyl jasmonate induced G_0/G_1 arrest in cultured SK-N-SH and BE(2)-C cells (Tables 1 and 2). These findings indicated that the methyl jasmonate-induced decrease in cell viability might be associated, at least in part, with downregulation of PCNA.

Methyl jasmonate induced apoptosis in neuroblastoma cells

Earlier studies demonstrated that inducing apoptosis was one of the mechanisms of antitumor effects of methyl jasmonate [5,12,13]. We hypothesized that methyl jasmonate might also induce apoptosis in cultured neuroblastoma cells. As shown in Fig. 3a–d, AO/EB and Hoechst 33258 staining indicated that administrations of

Fig. 1



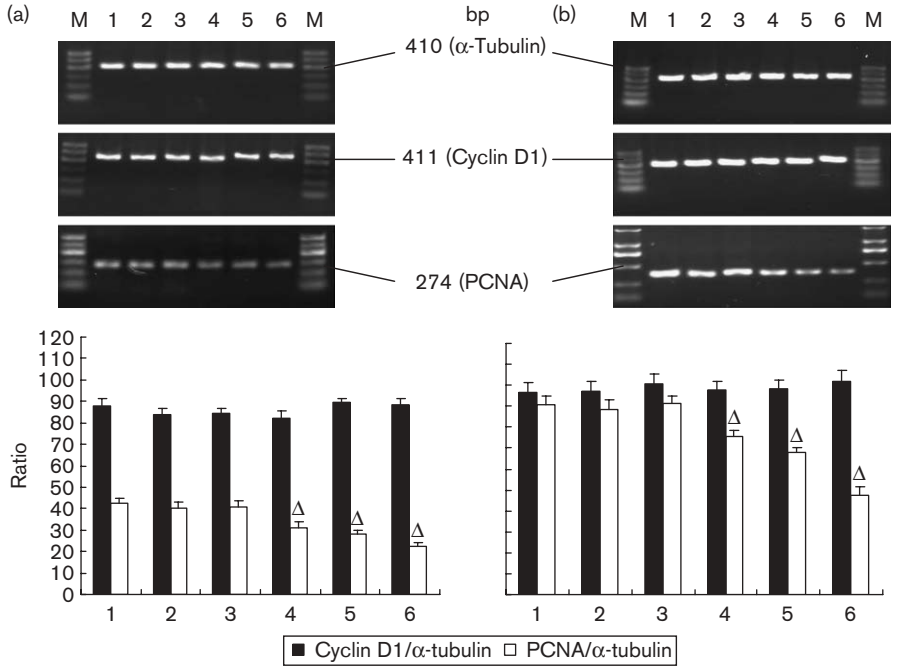
Methyl jasmonate suppresses the growth of neuroblastoma cells. Confluent monolayers of SK-N-SH and BE(2)-C cells were seeded into each well of 96-well or 6-well plates, and incubated with dimethyl sulfoxide (DMSO), methyl jasmonate or cisplatin as indicated. MTT colorimetric assay indicated that methyl jasmonate and cisplatin, but not DMSO, inhibited cell viability of SK-N-SH (a) and BE(2)-C (b) cells in a dose-dependent and time-dependent manner. Colony formation assay further demonstrated that administration of methyl jasmonate and cisplatin resulted in decreased cell proliferation of SK-N-SH (c) and BE(2)-C (d) cells. *Significant increase from control treated with DMSO ($P<0.05$). Triplicate experiments were performed with essentially identical results. From left to right: DMSO control, 1 mmol/l methyl jasmonate, 1.5 mmol/l methyl jasmonate, 2 mmol/l methyl jasmonate. 0.05 mmol/l cisplatin, 0.1 mmol/l cisplatin, and 0.25 mmol/l cisplatin.

methyl jasmonate and cisplatin, rather than DMSO, to SK-N-SH and BE(2)-C cells resulted in extensive DNA strand breakage and nuclei-shrunk, characteristic changes of apoptosis. These procedures and annexin V-FITC/PI staining flow cytometry were also used to quantify the number of apoptotic cells induced by methyl jasmonate. As shown in Fig. 3e and f, methyl jasmonate exerted strong apoptosis-inducing effects on neuroblastoma cells. The cellular apoptotic rates of methyl jasmonate-treated SK-N-SH and BE(2)-C cells were 21.36–43.73% and 33.5–75%, respectively. In addition, compared with apoptotic cells, the necrotic cells made up only a small section of the dead cells. The cellular necrotic rates of SK-N-SH and BE(2)-C cells induced by methyl jasmonate were 3.25–7.26% and 2.98–5.55%, respectively. These findings indicated that apoptosis was involved in methyl jasmonate-mediated anticancer activity on neuroblastoma cells.

Methyl jasmonate downregulated X-linked inhibitor of apoptosis protein and survivin in neuroblastoma cells

As previous evidence demonstrates that the inhibitors of IAPs play a central role in the regulation of apoptosis of tumor cells [22], we hypothesized that modulation of IAPs expression might participate in jasmonate-induced apoptosis of neuroblastoma cells. To meet this end, we performed RT-PCR to measure the expression of XIAP and survivin, the important members of IAP family. As

Fig. 2



Methyl jasmonate downregulates proliferating cell nuclear antigen (PCNA) expression of neuroblastoma cells. Confluent monolayers of SK-N-SH and BE(2)-C cells were treated with dimethyl sulfoxide (DMSO), methyl jasmonate or cisplatin for 24 h as indicated. RT-PCR demonstrated that methyl jasmonate, but not DMSO or cisplatin, decreased PCNA mRNA in SK-N-SH (a) and BE(2)-C (b) cells in a dose-dependent manner. Administration of methyl jasmonate, however, resulted in no changes in cyclin D1 mRNA levels. Δ indicates significant decrease from control treated with DMSO ($P<0.05$). Triplicate experiments were performed with essentially identical results. M, PCR marker (100–600 bp); 1, negative control; 2, DMSO control; 3, 0.1 mmol/l cisplatin; 4, 1.0 mmol/l methyl jasmonate; 5, 1.5 mmol/l methyl jasmonate; 6, 2.0 mmol/l methyl jasmonate.

Table 1 Cell cycle phases of SK-N-SH cells treated by methyl jasmonate for 24 h ($X \pm S$, $n=3$)

Group	G_0/G_1(%)	S (%)	G_2/M (%)
Negative control	53.32 ± 2.19	34.23 ± 1.38	12.45 ± 1.07
5 mmol/l DMSO	53.36 ± 0.54	33.89 ± 0.32	12.75 ± 0.85
1 mmol/l MJ	78.98 ± 1.04*	10.53 ± 0.60**	10.49 ± 1.61
1.5 mmol/l MJ	79.01 ± 1.18*	9.15 ± 0.79**	11.84 ± 1.02
2 mmol/l MJ	84.03 ± 1.15**	5.66 ± 0.20**	10.31 ± 1.04
0.1 mmol/l DDP	82.87 ± 1.04**	6.07 ± 0.31**	11.06 ± 0.91

DDP, cisplatin; DMSO, dimethyl sulfoxide; MJ, methyl jasmonate.
*$P<0.05$ versus control group; **$P<0.01$ versus control group.

Table 2 Cell cycle phases of BE(2)-C cells treated by methyl jasmonate for 24 h ($X \pm S$, $n=3$)

Group	G_0/G_1(%)	S (%)	G_2/M (%)
Negative control	63.50 ± 2.79	25.77 ± 1.24	10.73 ± 3.53
5 mmol/l DMSO	60.91 ± 2.57	28.52 ± 1.52	10.57 ± 3.40
1 mmol/l MJ	65.63 ± 2.91*	23.76 ± 1.30*	10.61 ± 2.50*
1.5 mmol/l MJ	69.10 ± 1.83*	19.47 ± 1.78**	11.43 ± 3.11
2 mmol/l MJ	72.16 ± 3.39**	15.68 ± 1.62**	12.16 ± 1.93
0.1 mmol/l DDP	71.47 ± 1.54**	14.95 ± 1.72**	13.58 ± 2.06

DDP, cisplatin; DMSO, dimethyl sulfoxide; MJ, methyl jasmonate.
*$P<0.05$ versus control group; **$P<0.01$ versus control group.

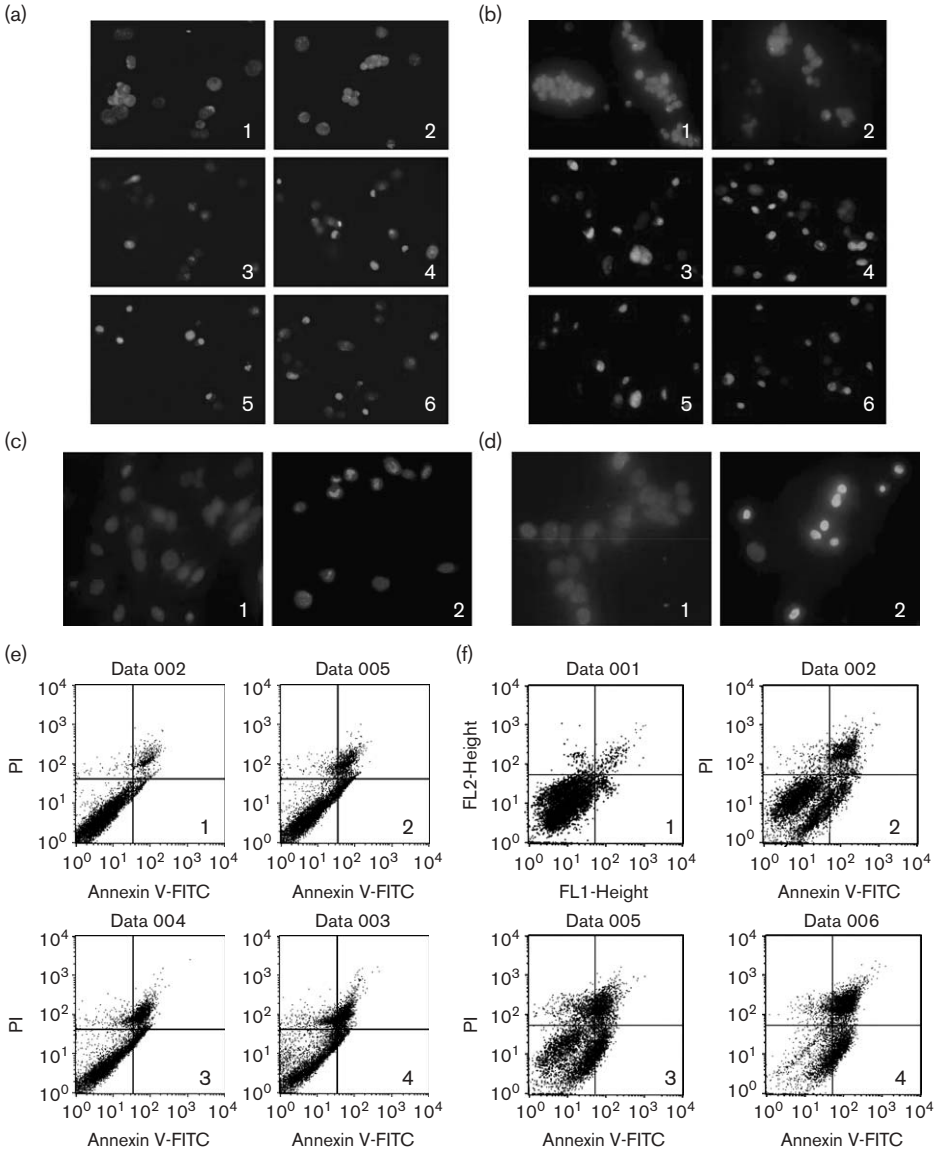
shown in Fig. 4a and b, methyl jasmonate and cisplatin, but not DMSO, attenuated the expression of XIAP and survivin via a dose-dependent manner in both SK-N-SH and BE(2)-C cells. These results indicated that downregulation of XIAP and survivin may be associated with methyl jasmonate-induced apoptosis in neuroblastoma cells.

Discussion

Neuroblastoma derived from primitive cells of the sympathetic nervous system is one of the most common solid tumors in childhood, characterized by two extreme disease courses, spontaneous regression, and life-threatening progression [23,24]. The clinical outcome is associated with disease stage, age at diagnosis, histologic classification, N-myc amplification, DNA ploidy, and TrkA overexpression [23–25]. These characteristics are therefore used to classify cases into low-risk, intermediate-risk, and high-risk groups. Despite treatment regimens including radiation therapy and high-dose chemotherapy with stem cell rescue, little progress has been made in improving the poor prognosis of patients with late-stage neuroblastoma because resistance to chemotherapy is common [25]. Therefore, new anticancer substances and treatment regimens are of interest.

Jasmonic acid and its methyl ester, methyl jasmonate, are fatty acid-derived cyclopentanones occurring throughout the plant kingdom and play major roles in defense against

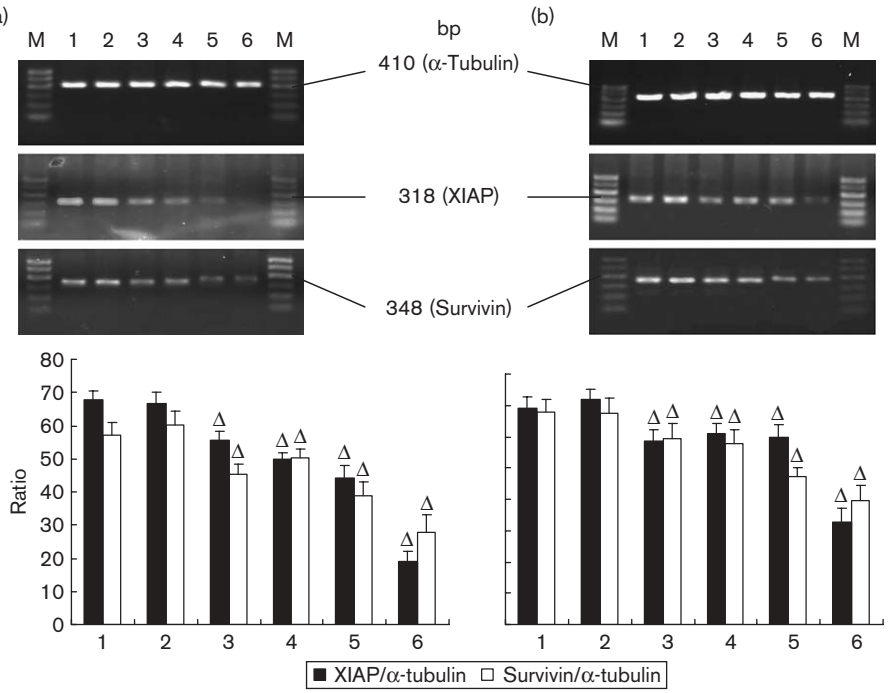
Fig. 3



Methyl jasmonate induces apoptosis in neuroblastoma cells. Confluent monolayers of SK-N-SH and BE(2)-C cells were incubated with dimethyl sulfoxide (DMSO), methyl jasmonate or cisplatin for 24 h as indicated. Cells were collected for acridine orange–ethidium bromide (AO/EB) staining, Hoechst 33258 staining, and annexin V-FITC/propidium iodide staining flow cytometry. AO/EB and Hoechst 33258 staining indicated that administrations of methyl jasmonate and cisplatin, rather than DMSO, to SK-N-SH (a and c) and BE(2)-C (b and d) cells, resulted in extensive DNA strand breakage and nuclei-shrunk, characteristic changes of apoptosis. ×200 magnification. The results from flow cytometry indicated that methyl jasmonate induced apoptosis in cultured SK-N-SH (e) and BE(2)-C (f) cells in a dose-dependent manner. Triplicate experiments were performed with essentially identical results. (a and b) 1, negative control; 2, DMSO control; 3, 1 mmol/l methyl jasmonate; 4, 1.5 mmol/l methyl jasmonate; 5, 2 mmol/l methyl jasmonate; 6, 0.1 mmol/l cisplatin. (c and d) 1, negative control; 2, 2 mmol/l methyl jasmonate. (e and f) 1, negative control; 2, 1 mmol/l methyl jasmonate; 3, 1.5 mmol/l methyl jasmonate; 4, 2 mmol/l methyl jasmonate.

insects and disease [26]. Structurally and biosynthetically jasmonates belong to the family of oxygenated fatty acid derivatives, oxylipins, which are produced via the oxidative metabolism of polyunsaturated fatty acids [27]. The structures of jasmonates are different from any group of currently available anticancer agents. Their activity and low levels of side effects usually encountered with existing cytotoxic drugs, position jasmonates as the very first of a new class of drug of major interest for oncologists and for their patients, whose chemotherapeutic promise should be realized through preclinical and clinical development [11]. Natural jasmonate derivatives, methyl jasmonate, jasmonic acid and *cis*-jasmone, have been compared as with their anticancer effects, using leukemia, lymphoma, breast, prostate and melanoma cancer cells as targets [5]. In general, methyl jasmonate has been found to be superior to

Fig. 4



Methyl jasmonate downregulates X-linked inhibitor of apoptosis protein (XIAP) and survivin expression in neuroblastoma cells. Confluent monolayers of SK-N-SH and BE(2)-C cells were stimulated with dimethyl sulfoxide (DMSO), methyl jasmonate or cisplatin for 24 h as indicated. RT-PCR demonstrated that methyl jasmonate decreased XIAP and survivin mRNA in SK-N-SH (a) and BE(2)-C (b) cells in a dose-dependent manner. In addition administration of cisplatin also resulted in decreased XIAP and survivin expression. DMSO administration, however, resulted in no changes in XIAP and survivin mRNA levels. Δ indicates significant decrease from control treated with DMSO ($P<0.05$). Triplicate experiments were performed with essentially identical results. M, PCR marker (100–600 bp); 1, negative control; 2, DMSO control; 3, 0.1 mmol/l cisplatin; 4, 1.0 mmol/l methyl jasmonate; 5, 1.5 mmol/l methyl jasmonate; 6, 2.0 mmol/l methyl jasmonate.

jasmonic acid in terms of cytotoxicity. Although samples from different chronic lymphocytic leukemia patients exhibit a spectrum of intensities in their response to jasmonates, their relative sensitivities can also be generally summed up as: methyl jasmonate > *cis*-jasmone > jasmonic acid [28]. Thus, the methyl group seems to contribute considerably to the cytotoxic effect of methyl jasmonate. In this study, we demonstrated that methyl jasmonate suppressed the growth of human neuroblastoma cell lines in a dose-dependent and time-dependent manner. Compared with cisplatin, methyl jasmonate seemed to affect neuroblastoma cells earlier but less effectively, which was consistent with previous reports that the direct effects of jasmonates on mitochondria might endow them with the ability to affect cancer cells earlier than common chemotherapeutic drugs [12,28]. Combined with the fact that jasmonates regulate stress responses in plants as well as in mammalian cancer cells [11], our findings suggest that identification of plant-derived substances with known roles in plant cell death may provide novel candidates for use in clinical oncology.

Cyclin D1, the founding member of the D-type cyclin family (cyclins D1, D2, and D3), is the cyclin that accumulates after mitogen stimulation [29]. It is widely assumed that cyclin D1 controls G_1 phase progression by binding and activating CDK4 and CDK6 [29]. Rearrangement, amplification, and/or increased expression of the cyclin D1 gene and overexpression of its mRNA have been reported in several types of human cancers [30]. Cyclin D1 downregulation correlates with the accumulation of cells in G_0/G_1 phase of cell cycle [31]. As recent evidence indicated that aspirin, also a plant stress hormone, induced downregulation of cyclin D1 and cell cycle arrest in cancer cells [32], and combing with our results that methyl jasmonate induced G_0/G_1 arrest in neuroblastoma cells, it was deemed of interest to investigate the role of cyclin D1 in the inhibitory effects of methyl jasmonate on human neuroblastoma cells. In this study, however, we found that administration of methyl jasmonate resulted in no changes in cyclin D1 levels, which indicates that some other mechanisms might be involved in methyl jasmonate-induced cell growth inhibition and cell cycle arrest.

PCNA is a protein that acts in conjunction with DNA polymerase δ during mitosis [33]. It also plays a central role in DNA replication, DNA repair, and cell cycle

progression [33]. Up to now, PCNA expression is an established operational marker for proliferating cells. A series studies have demonstrated that PCNA is overexpressed in many kinds of tumor tissues, and participates in the development and progression of cancer. We hypothesized that jasmonates might influence the PCNA expression of neuroblastoma cells. We found that jasmonates downregulated the PCNA expression of cultured neuroblastoma cells in a dose-dependent and time-dependent manner. Previous studies indicate that PCNA modulates cell cycle via combination with many kinds of cyclin-dependent kinase/cyclin complexes, and downregulation of PCNA via antisense oligonucleotides facilitates the cell cycle arrested at G_0/G_1 phase [34]. In this study, we found that methyl jasmonate induced G_0/G_1 arrest in neuroblastoma cells. We believe that this may be associated with downregulation of PCNA induced by methyl jasmonate.

Some research has demonstrated that the IAPs in mammal cells could suppress apoptosis through inhibiting procaspase activation and the catalytic activity of mature caspases [35]. The IAP family currently consists of five members: neuronal apoptosis inhibitory protein, XIAP, human inhibitor of apoptosis protein-1, human inhibitor of apoptosis protein-2, and survivin [36]. XIAP maps to the Xq24-25 region, an area that has been characterized by frequent clastogenic events, and is associated with a large number of cancers [37]. A series of studies has demonstrated that XIAP suppresses apoptosis via caspase-3 and caspase-7 inhibition. In neuroblastoma cells, XIAP protected against death induced by staurosporine, thapsigargin, or serum withdrawal [38], whereas downregulation of XIAP induced by amyloid-β attributed to increases in vulnerability to oxidative stress and apoptosis [39]. In primary neuroblastoma, survivin expression was associated with tumors of high risk and unfavorable prognosis, whereas proapoptotic receptor expression was more abundant in tumors of favorable prognosis [40]. It was indicated that survivin expression seemed to be more predictive of recurrent disease than N-myc amplification [40]. In neuroblastoma cell lines, survivin expression was associated with greater proliferation rates and greater resistance to drug-mediated or immune-mediated cell death [40]. Therefore, XIAP and survivin could be potential targets for the treatment of neuroblastoma. In this study, we found that methyl jasmonate induced apoptosis of neuroblastoma cells in a dose-dependent and time-dependent manner. Besides, the expression of XIAP and survivin was suppressed in methyl jasmonate-treated neuroblastoma cells. We believe that methyl jasmonate may modulate IAPs to exert anticancer activity, which warrants our further study.

In summary, in this study we demonstrated that methyl jasmonate suppressed cell viability of cultured neuroblastoma cells associated with downregulation of PCNA, and induced apoptosis accompanied by downregulation of XIAP and survivin. These findings lay the groundwork for further investigation into the mechanisms of methyl jasmonate-mediated anticancer function on neuroblastoma cells. The jasmonate family of novel anticancer agents presents new hope for the development of cancer therapeutics, which should attract further scientific and pharmaceutical interest. Potential future research directions include identification of the actual jasmonate target molecule(s) and structure–function analysis to discover new derivatives with superior therapeutic index.

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