Potentiation of Paclitaxel Cytotoxicity by Inostamycin in Human Small Cell Lung Carcinoma, Ms-1 Cells

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In the present study, we found that inostamycin increased the ability of paclitaxel to induce apoptosis in Ms-1 cells. A considerably higher concentration of paclitaxel was required for the induction of apoptosis in Ms-1 cells than in other cell lines tested. Treatment of Ms-1 cells with inostamycin, an inhibitor of phosphatidylinositol (PI) synthesis, reduced the dosage of paclitaxel required to induce cell death by apoptosis. This effect of inostamycin is specific to Ms-1 cells, and inostamycin did not increase the cytotoxicity of other antitumor drugs such as adriamycin, vinblastine, methotrexate, cisplatin, etoposide, or camptothecin in Ms-1 cells. Addition of inostamycin to paclitaxel-treated cells caused a significant increase in the sub G1 peak, representing apoptosis, which was accompanied by a decrease in the G2/M peak seen in paclitaxel-treated Ms-1 cells, without affecting paclitaxel-inhibited tubulin depolymerization. Moreover, paclitaxel did not enhance inostamycin-inhibited PI synthesis. The expression levels of Bcl-2, Bax, and Bcl-XL were not changed following the co-treatment with inostamycin plus paclitaxel, whereas the activated form of caspase-3 was markedly increased. Thus, inostamycin is a chemosensitizer of paclitaxel in small cell lung carcinoma Ms-1 cells.

Key words: Paclitaxel — Apoptosis — Small cell lung carcinoma — Inostamycin — Caspase-3

Lung cancer is the most common fatal malignancy in the developed world. Small cell lung carcinoma (SCLC) constitutes 25% of all lung cancers and follows an aggressive clinical course. In spite of initial sensitivity to radioand chemotherapy, the 2-year survival of patients with SCLC remains less than 5%.1) Thus, novel therapeutic strategies are required, and it is most likely that these strategies will arise from a better understanding of the cell biology of SCLC cells. One such novel approach could involve agents that interfere with the critical intracellular signal transduction pathways mediating cell survival.2) However, the efficacy of available anticancer drugs is not sufficient for treatment of most solid tumors and the usefulness of these drugs is, in any case, limited.

Paclitaxel is a terpenoid agent extracted from the bark of the tree, Taxus brevifolia. The drug has displayed significant antitumor efficacy against breast and ovarian cancers in clinical trials3, 4) and is being investigated as an active agent against many other cancers, including lung, head and neck, bladder, and lymphomas. Paclitaxel binds to and stabilizes microtubules, preventing depolymerization.5, 6) This results in G2/M arrest and apoptotic cell death. Among the growing number of genes known to be involved in regulating apoptosis is the bcl-2 family of genes. Bcl-2 and Bcl-Xi are members of the Bcl-2-related family and act to prevent apoptosis,7, 8) whereas Bax, another Bcl-2 family member, functions as a death agonist.9) Recently, it has been shown that paclitaxel induces Bcl-2 phosphorylation, which is accompanied with loss of function. On the other hand, it has been demonstrated that, although different anticancer agents affect diverse intracellular targets at cytocidal concentrations, they eventually trigger the cleavage and the activation of the interleukin-1β converting enzyme (ICE)/CED-3 family of proteases (now called caspases), of which caspase-3 is a key member.10, 11) The active mature form of caspase-3 is composed of a heterodimer of p17 and p12.12) Although it is not known how these proteases are selectively activated by treatment with a variety of drugs, the upstream activation mechanism for caspase-3 may be an extremely attractive target for therapeutic modulation.

Inostamycin, a novel polyether compound, was originally isolated from Streptomyces sp. MH816-AF15 as an inhibitor of CDP-DG:inositol transferase, which catalyzes phosphatidylinositol (PI) synthesis.13, 14) It does not directly inhibit PI-specific phospholipase C, PI kinase, protein kinase C, mitogen-activated protein kinase, casein kinase II, CDK2 and tyrosine kinases or macromolecular synthesis.14–16) Inostamycin induced arrest of cell growth at the G1 phase of the cell cycle, inhibiting the expression

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of cyclin D1 and causing accumulation of p27KIP1 in SCLC Ms-1 cells.

In the present study, we demonstrated that inostamycin markedly enhanced the cytotoxicity of paclitaxel through the activation of caspase-3 in SCLC cells.

MATERIALS AND METHODS

Materials
Inostamycin was isolated from Streptomyces as described previously.13) Hoechst 33258 was purchased from Polyscience. Paclitaxel, adriamycin, etoposide, methotrexate, camptothecin, and cis-platinum (II) diammine dichloride (cisplatin) were obtained from Sigma. Vinblastine was from Wako Pure Chemicals (Osaka).

Cell culture
Human SCLC cell line Ms-1,17, 18) human esophageal cancer cell line TE-3,19) and human carcinoma cell line KB3-1 were cultured in RPMI 1640 containing 5% fetal bovine serum, penicillin G (100 units/ml), and kanamycin (0.1 mg/ml) at 37°C in a 5% CO2-95% air atmosphere.

Cell growth assay
Ms-1 cells (2×10⁴) were plated in 100 µl of growth medium in 96-well plates, and incubated at 37°C for 24 h. Anticancer drugs and inostamycin were added, and the cells were then further incubated for 2 days. Ten microliters of 5 mg/ml 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT) in phosphate-buffered saline (PBS) was added to each well, and the plate was incubated at 37°C for 4 h. Then, 100 µl of isopropanol containing 0.04 N HCl was added and mixed thoroughly. Formazan formation was measured at 570 nm for the sample and at 620 nm for the reference by using a micro plate reader MPR-A4 (Tosho). The blank value measured in the absence of cells was subtracted from all the data.

Hoechst 33258 staining
Ms-1 cells were fixed with 3% paraformaldehyde for 20 min at room temperature.

Fig. 1. Enhancement of paclitaxel-induced cytotoxicity by inostamycin in Ms-1 cells. Ms-1 cells (A) or KB3-1 (B) cells were incubated with various concentrations of anticancer drugs in the presence of 0 (○), 0.01 (△), 0.03 (□), or 0.1 (●) µg/ml inostamycin for 2 days. At the end of this incubation, the 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT) assay for relative cell growth was used, as described in “Materials and Methods.”
washed with distilled water, and dried. Then, the cells were stained with Hoechst 33258 (10 µg/ml) for 5 min, washed, and examined by fluorescence microscopy.

**Flow cytometry** The cells were washed twice with PBS and then fixed in 80% ethanol at 4°C. After having been washed again twice with PBS, the fixed cells were resuspended in 0.2 mg/ml propidium iodide containing 0.6% NP-40 and 1 mg/ml RNase A and incubated for 1 h at room temperature in the dark. The cell suspensions were analyzed in a flow cytometer (Epics Elite).

**Tubulin depolymerization assay** Ms-1 cells were treated with chemicals for 12 h. They were then washed and lysed in hypotonic buffer (20 mM Tris-HCl, 1 mM MgCl₂, 2 mM EGTA, 0.5% NP-40, 2 mM phenylmethylsulfonyl fluoride [PMSF], and 0.1 µg/ml vinblastine, pH 6.8). Cell lysates were centrifuged at 14,000 rpm for 10 min at room temperature. Supernatants containing soluble tubulin were carefully separated from pellets containing polymerized tubulin. Western blot analysis for α-tubulin was performed.

**Incorporation of ³²Pi into phospholipids** Ms-1 cells were labeled with ³²Pi (5 µCi/ml) in phosphate-free RPMI 1640 medium for 4 h. The medium was then removed, and 0.5 ml of 10% trichloroacetic acid was added to the

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**Fig. 2.** Inhibition of Ms-1 cell growth by treatment with inostamycin and paclitaxel. Ms-1 cells were treated with paclitaxel (1 µg/ml) and/or inostamycin (0.1 µg/ml) for the indicated times. o none, • inostamycin, ▲ paclitaxel, ■ inostamycin plus paclitaxel. Relative cell growth was assessed by means of the MTT assay, as described in “Materials and Methods.”

**Fig. 3.** Induction of apoptosis by treatment with inostamycin and paclitaxel in Ms-1 cells. Ms-1 cells were left untreated (a), treated with 0.1 µg/ml inostamycin (b), 1 µg/ml paclitaxel (c), or 0.1 µg/ml inostamycin and 1 µg/ml paclitaxel (d) for 12 h. Then, the cells were fixed, stained with Hoechst 33258, and observed under a fluorescence microscope.
cells. The cells were scraped off the dishes, and the lipids were extracted with chloroform-methanol. Lipid extracts labeled with $^{32}$Pi were spotted on a silica gel thin-layer chromatography plate, which was developed with chloroform-methanol-acetic acid-H$_2$O (25:15:4:2). Radioactive spots were identified by autoradiography.

**Western blotting** The cells were lysed in lysis buffer (50 mM HEPES, 150 mM NaCl, 2.5 mM EDTA, 1 mM dithiothreitol [DTT], 10 mM β-glycerophosphate, 1 mM NaF, 0.1 mM Na$_3$VO$_4$, 0.1% Tween 20, 10% glycerol, 1 mM phenylmethanesulfonyl fluoride [PMSF], 10 µg/ml leupeptin; pH 7.5), and then sonicated twice for 10 s on ice. Loading buffer (42 mM Tris-HCl, 10% glycerol, 2.3% sodium dodecyl sulfate [SDS], 5% 2-mercaptoethanol, and 0.002% bromophenol blue; pH 6.8) was then added to each lysate, which was subsequently boiled for 3 min and electrophoresed on an SDS-polyacrylamide gel. Proteins were transferred to Hybond-P membranes (Amersham), and immunoblotted with antibodies against CPP32 (caspase-3), Bcl-X$_L$, Bax (Santa Cruz), Bcl-2 (124; DAKO), cyclin D1 (UBI), or α-tubulin (Sigma). Detection was performed with enhanced chemiluminescence reagent (DuPont).

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Fig. 4. Effect of inostamycin and/or paclitaxel on cell cycle distribution of Ms-1 cells. Ms-1 cells were left untreated (a), treated with 0.1 µg/ml inostamycin (b), 1 µg/ml paclitaxel (c), or 0.1 µg/ml inostamycin and 1 µg/ml paclitaxel (d) for 24 h. Then, the cells were collected, and their DNA content was analyzed by flow cytometry.

Fig. 5. (A) Effect of inostamycin on paclitaxel-inhibited tubulin depolymerization. Ms-1 cells were treated or not with 1 µg/ml paclitaxel in the presence or absence of 0.1 µg/ml inostamycin for 12 h. The cells were then lysed and the soluble (S) and insoluble (P) fractions were separated, as described in “Materials and Methods.” (B) Ms-1 cells were treated or not with 0.1 µg/ml inostamycin in the presence or absence of paclitaxel (1 µg/ml) for 4 h in $^{32}$P-containing RPMI 1640 medium. The lipids were then extracted and analyzed by thin-layer chromatography, as described in “Materials and Methods.” Lipid standards were visualized with I$_2$ vapor. PC, phosphatidylycerol; PE, phosphatidylethanolamine.
RESULTS

Enhancement of paclitaxel-induced cytotoxicity by inostamycin in Ms-1 cells  As shown in Fig. 1A, paclitaxel inhibited the growth of Ms-1 cells with an IC\textsubscript{50} of 0.2 µg/ml, whereas it did so with an IC\textsubscript{50} of 0.0, 0.005, or 0.0002 µg/ml in the presence of inostamycin at 0.01, 0.03 or 0.1 µg/ml, respectively. However, inostamycin only slightly increased etoposide cytotoxicity at 0.1 µg/ml, and did not increase the cytotoxicity of adriamycin, vinblastine, methotrexate, cisplatin, or camptothecin in Ms-1 cells (Fig. 1A). Inostamycin did not potentiate the cytotoxic effect of paclitaxel in other cancer cell lines, KB3-1 and TE-3 cells (Fig. 1B and data not shown). Thus, it is suggested that potentiation of the antiproliferative effect of paclitaxel by inostamycin is specific to Ms-1 cells.

Induction of apoptosis by paclitaxel in inostamycin-treated Ms-1 cells  Inostamycin (0.1 µg/ml), as well as paclitaxel (1 µg/ml), suppressed the growth of Ms-1 cells, but did not induce cell death, at least up to 3 days. However, treatment of Ms-1 cells with inostamycin plus paclitaxel gradually decreased the cell number of Ms-1 cells (Fig. 2). This decreased cell number was due to induction of apoptosis, because after 12 h in culture, nuclear condensation and fragmentation were observed in paclitaxel plus inostamycin-treated Ms-1 cells, whereas either drug alone did not induce apoptotic nuclear changes (Fig. 3).

As shown in Fig. 4, flow-cytometric DNA content analysis demonstrated that 24 h of exposure to paclitaxel (1 µg/ml) caused significant accumulation of cells in the G2/M phase of the cell cycle, and inostamycin (0.1 µg/ml) increased the population of cells in G1. It is noteworthy that treatment of Ms-1 cells with paclitaxel (1 µg/ml) plus inostamycin (0.1 µg/ml) for 24 h caused a significant increase in the sub G1 peak (46%), representing apoptosis, which was accompanied by a decrease in the population of cells in G2 compared with that following treatment with paclitaxel alone or by a decrease in that in G1 compared with that following treatment with inostamycin alone. Next we examined whether inostamycin enhanced paclitaxel-inhibited tubulin depolymerization in Ms-1 cells. As shown in Fig. 5A, treatment of Ms-1 cells with paclitaxel (1 µg/ml) caused tubulin polymerization, resulting in a shift in tubulin from the unpolymerized state found in the cell supernatant to the polymerized state found in the cell pellet, even in the presence of inostamycin (0.1 µg/ml). Moreover, paclitaxel did not enhance inostamycin-inhibited PI synthesis (Fig. 5B). Therefore, these results suggest that this synergistic effect may not be due to enhancement of the accumulation of each drug.

Effect of inostamycin and paclitaxel on Bcl-2, Bax, Bcl-X\textsubscript{L}, cyclin D1, and caspase-3 expression in Ms-1 cells  Because treatment with paclitaxel is known to induce Bcl-2 phosphorylation, producing an inactive form of Bcl-2, in a variety of human cancer cell lines,\textsuperscript{20, 21} we examined the effect of inostamycin on paclitaxel-induced Bcl-2 phosphorylation. Paclitaxel (1 µg/ml)-induced Bcl-2 phosphorylation was not enhanced in the presence of 0.1 µg/ml of inostamycin (Fig. 6). Furthermore, treatment of Ms-1 cells with inostamycin and/or paclitaxel did not change the expression levels of other Bcl-2 family proteins, Bax and Bcl-X\textsubscript{L} (Fig. 6). Previously, we reported that inostamycin (0.1 µg/ml) lowers the expression levels of cyclin D1.\textsuperscript{22} Paclitaxel affected neither cyclin D1 expression nor inostamycin-inhibited cyclin D1 expression.

The proteolytic cleavage of pro-caspase-3 (p32) to produce two subunits, p17 and p12,\textsuperscript{23} is essential for the formation of active caspase-3. There was increased expression of the p17 caspase-3 subunit after treatment with inostamycin (0.1 µg/ml) plus paclitaxel (1 µg/ml) under conditions where each drug induced slight activation of caspase-3 (Fig. 6).

DISCUSSION

SCLC is a disease in which treatment with standard combination chemotherapy can lead to response rates of greater than 80%, but paradoxically not result in significant improvement of long-term survival. Therefore, effective treatment of this disease will depend on the
development of new drugs and therapeutic approaches. This study describes the effectiveness of combination therapy with inostamycin and paclitaxel for inhibition of cell growth and induction of apoptosis in SCLC Ms-1 cells. SCLC Ms-1 cells were less sensitive to paclitaxel than non-SCLC cell lines. In all cells we tested, inostamycin induced G1 arrest and paclitaxel induced G2/M blocks. However, potentiation of paclitaxel cytotoxicity by inostamycin is specific to Ms-1 cells. Since inostamycin does not potentiate the cytotoxicity of another G2/M blocker, vinblastine, in Ms-1 cells, it is not likely that the potentiation of paclitaxel by inostamycin is due to the combination of G2/M and G1 blockers. We don’t know at present why inostamycin potentiated the cytotoxic effect of paclitaxel. It was reported that inostamycin reverses multidrug resistance in human carcinoma KB-C4 cells. However, verapamil, which is known to overcome multidrug resistance, did not change the susceptibility to paclitaxel in Ms-1 cells (data not shown). Furthermore, Ms-1 cells are not resistant to multidrug resistance (MDR) protein-sensitive chemotherapeutic drugs including adriamycin. Therefore, it is unlikely that Ms-1 cells express MDR proteins and inostamycin circumvents multidrug resistance in Ms-1 cells. The failure to undergo apoptosis upon paclitaxel treatment could be caused by inactivation of signaling pathways leading to the execution of apoptosis in Ms-1 cells. Therefore inostamycin might modulate the inactivation mechanism of paclitaxel-generated apoptotic signals in Ms-1 cells. Bcl-2 family proteins are known to be involved in the regulation of apoptotic signals. However, inostamycin did not change the expression levels of Bcl-2 family proteins, such as Bcl-2, Bcl-XL, and Bax. It has been shown that paclitaxel inhibits microtubule depolymerization in the G2/M phase of the cell cycle and that this tubulin damage induces Bcl-2 phosphorylation, followed by apoptosis. Phosphorylation of Bcl-2 at serine residues leads to loss of Bcl-2 anti-apoptotic function. However, inostamycin enhanced neither paclitaxel-induced nor paclitaxel-induced Bcl-2 phosphorylation, suggesting that inostamycin exerted its effect downstream of Bcl-2 in Ms-1 cells. Another essential component of the apoptotic machinery is caspase-3, which is thought to function downstream of Bcl-2 family proteins. We found that co-treatment with inostamycin plus paclitaxel markedly induced caspase-3 activation under conditions where each drug alone failed to induce fully caspase-3 activation. These findings suggest that inostamycin modulates the paclitaxel-induced signals leading to caspase-3 activation, thereby accelerating the progression of the cells to apoptosis. Therefore, inostamycin may be a novel chemosensitizer for paclitaxel in a subset of SCLC cells.

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REFERENCES

1) Smyth, J. F., Fowlie, S. M., Gregor, A., Crompton, G. K., Busuttil, A., Leonard, R. C. F. and Grant, I. W. B. The impact of chemotherapy on small cell carcinoma of bronchus. Q. J. Med., 61, 969–976 (1986).
2) Levitski, A. and Gazit, A. Tyrosine kinase inhibition; an approach to drug development. Science, 267, 1782–1788 (1995).
3) Rowinsky, E. K., Cazenave, L. A. and Donehower, R. C. Taxol: a novel investigational antimitotubule agent. J. Natl. Cancer Inst., 82, 1247–1259 (1990).
4) Gelmon, K. A. Biweekly paclitaxel (taxol) and cisplatin in breast and ovarian cancer. Semin. Oncol., 21, 24–28 (1994).
5) Parness, J. and Horwitz, S. B. Taxol binds to polymerized tubulin in vitro. J. Cell Biol., 91, 479–487 (1981).
6) Horwitz, S. B. Mechanism of action of Taxol. Trends Pharmacol. Sci., 13, 134–136 (1992).
7) Reed, J. C. Bcl-2 and the regulation of programmed cell death. J. Cell Biol., 124, 1–6 (1994).
8) Boise, L., Gonzalez-Garcia, M., Postema, C., Ding, L., Lindsten, T., Turka, L., Mao, X., Nunez, G. and Thompson, C. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell, 74, 597–608 (1993).
9) Oltval, Z. N., Milliman, C. L. and Korsmeyer, S. J. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell, 74, 609–619 (1993).
10) Alnemri, E. S., Livingston, D., Nicholson, D. W., Salvesen, G., Thornberry, N. A., Wong, W. W. and Yuan, J. Human ICE/CED-3 protease nomenclature. Cell, 87, 171 (1996).
11) Chen, Z., Naito, M., Mashima, T. and Tsuruo, T. Activation of actin-cleavable interleukin 1β-convertase enzyme (ICE) family protease CPP-32 during chemotherapeutic agent-induced apoptosis in ovarian carcinoma cells. Cancer Res., 56, 5224–5229 (1996).
12) Nicholson, D. W. ICE/CED3-like proteases as therapeutic targets for the control of inappropriate apoptosis. Nat. Biotechnol., 14, 297–301 (1996).
13) Inoto, M., Umezawa, K., Takahashi, Y., Naganawa, H., Itaka, Y., Nakamura, H., Koizumi, Y., Sasaki, Y., Hamada, M., Sawa, T. and Takeuchi, T. Isolation and structure determination of inostamycin, a novel inhibitor of phosphatidylinositol turnover. J. Nat. Prod., 53, 825–829
14) Imoto, M., Taniguchi, Y. and Umezawa, K. Inhibition of CDP-DG:inositol transferase by inostamycin. *J. Biochem.*, **112**, 299–302 (1992).

15) Imoto, M., Morii, T., Deguchi, A. and Umezawa, K. Involvement of phosphatidylinositol synthesis in the regulation of S phase induction. *Exp. Cell Res.*, **215**, 228–233 (1994).

16) Deguchi, A., Imoto, M. and Umezawa, K. Inhibition of G1 cyclin expression in normal rat kidney cells by inostamycin, a phosphatidylinositol synthesis inhibitor. *J. Biochem.*, **120**, 1118–1122 (1996).

17) Kudoh, S., Takada, M., Masuda, N., Nakagawa, K., Itoh, K., Kusunoki, Y., Negoro, S., Matsui, K., Takifuji, N., Morino, H. and Fukuoka, M. Enhanced antitumor efficacy of a combination of CPT-11, a new derivative of camptothecin, and cisplatin against human lung tumor xenografts. *Jpn. J. Cancer Res.*, **84**, 203–207 (1993).

18) Tamura, K., Takada, M., Kawase, I., Tada, T., Kudoh, S., Okishio, K., Fukuoka, M., Yamaoka, N., Fujiwara, Y. and Yamakido, M. Enhancement of tumor radio-response by irinotecan in human lung tumor xenografts. *Jpn. J. Cancer Res.*, **88**, 218–223 (1997).

19) Doki, Y., Imoto, M., Han, E. K. H., Sgambato, A. and Weinstein, I. B. Increased expression of the p27<sup>KIP1</sup> protein in human esophageal cancer cell lines that over-express cyclin D1. *Carcinogenesis*, **18**, 1139–1148 (1997).

20) Haldar, A., Basu, A. and Croce, C. M. Bcl2 is the guardian of microtubule integrity. *Cancer Res.*, **57**, 229–233 (1997).

21) Haldar, A., Chintapalli, J. and Croce, C. M. Taxol-induced bcl-2 phosphorylation and death of prostate cancer cells. *Cancer Res.*, **56**, 1253–1255 (1996).

22) Imoto, M., Tanabe, K., Simizu, S., Tashiro, E., Takada, M. and Umezawa, K. Inhibition of cyclin D1 expression and induction of apoptosis by inostamycin in small cell lung carcinoma cells. *Jpn. J. Cancer Res.*, **89**, 315–322 (1998).

23) Kawada, M. and Umezawa, K. Long-lasting accumulation of vinblastine in inostamycin-treated multidrug-resistant KB cells. *Jpn. J. Cancer Res.*, **82**, 1160–1164 (1991).

24) Yusa, K. and Tsuruo, T. Reversal mechanism of multidrug resistance by verapamil: direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells. *Cancer Res.*, **49**, 5002–5006 (1989).

25) Haldar, S., Jena, N. and Croce, C. M. Inactivation of bcl-2 by phosphorylation. *Proc. Natl. Acad. Sci. USA*, **92**, 4507–4511 (1995).

26) Shimizu, S., Eguchi, Y., Kamiike, W., Matsuda, H. and Tsujimoto, Y. Bcl-2 expression prevents activation of the ICE protease cascade. *Oncogene*, **12**, 2251–2257 (1995).

27) Ibrado, A. M., Huang, Y., Fang, G., Liu, L. and Bhalla, K. Overexpression of Bcl-2 or Bcl-X<sub>L</sub> inhibits Ara-C-induced CPP32/Yama protease activity and apoptosis of human acute myelogenous leukemia HL-60 cells. *Cancer Res.*, **56**, 4743–4748 (1996).