EAT/mcl-1, a Member of the bcl-2 Related Genes, Confers Resistance to Apoptosis Induced by \textit{cis}-Diammine Dichloroplatinum (II) via a p53-Independent Pathway

Takashi Ando,1,2 Akihiko Umezawa,1 Atsushi Suzuki,1 Hajime Okita,1 Makoto Sano,1 Yoshiki Hiraoka,3 Sadakazu Aiso,3 Takao Saruta2 and Jun-ichi Hata1,4

1Department of Pathology, 2Internal Medicine and 3Anatomy, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582

EAT/mcl-1 showed increased expression during the differentiation of a multipotent human embryonic carcinoma cell line, NCR-G3, and of myeloblastic cells “ML-1,” and has sequence similarity to Bcl-2. In this present study, we determined whether the apoptotic cell death induced by chemotherapeutic agents could be inhibited by EAT/mcl-1, as has been found with Bcl-2. Cells transfected with EAT/mcl-1 showed higher resistance to \textit{cis}-diammine dichloroplatinum (II) (CDDP) and carboplatin compared with the parental line (10)1 and neomycin-resistance gene-transfected clone, (10)1/neo. There was, however, no difference in sensitivity to etoposide, N,N′-bis-(2-chloroethyl)-N′-(3-hydroxypropyl) phosphorodiamic acid cyclic ester monohydrate, adriamycin or other chemotherapeutic agents tested. DNA fragmentation of the parental cells following treatment with CDDP and carboplatin was observed in a concentration-dependent manner. In contrast, cells transfected with EAT/mcl-1 did not show DNA fragmentation following treatment with the same concentration of these drugs. EAT/mcl-1 was capable of delaying the onset of p53-independent apoptosis, although it could not inhibit apoptosis completely. Since CDDP and carboplatin damage DNA and then activate c-abl and the JNK/SAPK pathway, EAT/mcl-1 may inhibit p53-independent apoptosis through a c-abl/JNK (SAPK)-dependent mechanism. EAT/mcl-1 has functional homology to Bcl-2 in that it can enhance cell viability under conditions which otherwise cause apoptosis and increase resistance to chemotherapeutic agents.

Key words: mcl-1/EAT — Apoptosis — \textit{cis}-Diammine dichloroplatinum (II) — bcl-2 — p53

We previously established a multipotent human embryonic carcinoma (EC) cell line, NCR-G3, to serve as a model system of early human embryogenesis.1) NCR-G3, which is derived from a testicular EC, possesses pluripotency in differentiation, and is capable of differentiation to trophoderm cells upon exposure to retinoic acid (RA) or heat shock.2,3) These differentiated cells produce human chorionic gonadotropin, a trophoderm-specific hormone.3) We employed this system to search for molecules essential for EC cell differentiation. EAT/mcl-1 gene was found to be one such gene; it is up-regulated during the early stage of EC cell differentiation.1) EAT/mcl-1 gene was originally identified as a gene which is induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) in myeloid leukemia cells.6) In both cell types, gene expression is increased at the early stages of differentiation upon exposure to inducers.

Since its discovery, EAT/mcl-1 has attracted much interest from various researchers because of its sequence similarity to bcl-2. This group of genes differs from many oncogenes in that it can promote cell survival or inhibit apoptosis without promoting cell proliferation.5) The ability of Bcl-2 to inhibit apoptosis has been observed in many different cell types, as well as under a variety of conditions, including incubation of sensitive cells with glucocorticoids, removal of required growth factors, or exposure to cytotoxic drugs, γ-radiation or apoptosis-inducing gene products such as c-Myc.6–8) Several other proteins with sequence similarity to Bcl-2 can also influence cell survival. Bcl-xL was isolated by low stringency hybridization using \textit{bcl-2} gene as a probe; Bcl-xL is even more efficacious than Bcl-2 in promoting cell survival under certain apoptosis-inducing conditions.9) Another family member, Bax, was discovered as a gene product that can heterodimerize with Bcl-210); the heterodimer appears to protect cells, whereas Bax homodimers cause accelerated apoptosis.11) Related proteins have been identified in other species, all of which have sequence similarity to the carboxyl region of Bcl-2. The CED-9 protein of \textit{Caenorhabditis elegans} prevents programmed cell death during development of this nematode.12) The Epstein-Barr virus protein BHRF1 can also enhance cell viability.13,14) Considering the sequence similarity between EAT/mcl-1 and this Bcl-2 family, it is speculated that EAT/mcl-1 might also influence cell viability. A previous study has

4 To whom correspondence should be addressed.
shown that apoptosis, induced by c-myc overexpression in Chinese Hamster Ovary (CHO) cell line, was delayed by EAT/mcl-1.\(^{15}\) Apoptosis induced by etoposide was also reported to be inhibited by EAT/mcl-1 in FDP-C1, murine myeloid progenitor cells.\(^{16}\) However, little is known about the mechanism by which EAT/mcl-1 contributes to apoptosis. Based on the studies so far reported, several apoptotic pathways have been postulated.\(^{17}\) Although apoptosis and its inhibition by Bcl-2 have been well documented,\(^{18-22}\) similar mechanisms involving EAT/mcl-1 have not been fully elucidated. In this study, we examined whether EAT/mcl-1 overexpression could affect apoptosis induced by chemotherapeutic agents such as cis-diammine dichloroplatinum (II) (CDDP), alkylating agents, antibiotics and topoisomerase inhibitors in (10)\(^1\) mouse fibroblast cell line.\(^{23}\)

**MATERIALS AND METHODS**

**Cell line and culture** Mouse p53-deficient (10)\(^1\) fibroblast cell line\(^{23}\) was grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and incubated at 37°C in a humidified atmosphere of 5% CO\(_2\) and 95% air.\(^{22}\)

**Plasmids** A cDNA clone containing the entire EAT/mcl-1 coding region was excised with PsiI and ApaI from the Bluescript SK(–) vector and blunted. After addition of PsiI linker and digestion with PsiI, the 2.4-kb fragment obtained was inserted into the PsiI site of the pSR\(\alpha\)-neo vector. This places EAT/mcl-1 under the SR\(\alpha\) promoter. The plasmid pSR\(\alpha\)-neo was constructed by cloning the neomycin resistance gene-containing fragment into EcoRI/HindIII-cut pBR322-SR\(\alpha\).

**DNA transfection and amplification** Cells were transfected by the Lipofectin method (Gibco BRL, Gaithersburg, MD) according to the instructions of the manufacturer. (10)\(^1\) cells were plated at a density of 2×10\(^5\) cells/60 mm dish and grown overnight. Five micrograms of pSR\(\alpha\)-neo with or without 0.5 \(\mu\)g of pSR\(\alpha\)-EAT/mcl-1 and 10 \(\mu\)g of Lipofectin reagents were added to cells and incubation was continued for 48 h. Cells were grown at a concentration of 600 \(\mu\)g/ml of G418 for approximately 2 weeks.

**Immunoblotting assay** Immunoblot analysis for EAT/mcl-1 protein was carried out as previously described.\(^{24}\) Preblocked blots were reacted with rabbit polyclonal antibody against human EAT/mcl-1 (Pharmingen, San Diego, CA) at a 1:2000 dilution in Tris-buffered saline (pH 7.6) with 0.05% Tween 20 (TBS-T) at room temperature for 1 h and then incubated with a 1:2000 dilution of peroxidase-conjugated swine anti-rabbit IgG antibody (Zymed, San Francisco, CA) in TBS-T for 1 h. For immunocytochemistry, we generated a monoclonal antibody, 3A2. The 3A2 monoclonal antibody specifically reacts with human EAT/mcl-1. Immunoblots were developed by using the ECL western blotting detection system (Amersham International, Amersham, UK). Prestained molecular weight markers (“Rainbow” proteins) were obtained from Amersham.

**DNA fragmentation in (10)\(^1\) cells** (10)\(^1\) cells (5×10\(^6\) cells) were harvested and suspended in 200 \(\mu\)l of DNA extraction buffer (10 mM Tris-HCl [pH 7.4], 10 mM EDTA, 0.5% Triton X-100, 0.4 mg/ml RNase A). After incubation at 37°C for 1 h, a 2-\(\mu\)l aliquot of 20 mg/ml proteinase K was added and the mixture was further incubated for an additional hour. Then the sample was analyzed by electrophoresis in 2% agarose gels followed by ethidium bromide staining. The stained gels were photographed under a UV illuminator.

**RNA blot analysis** RNA was prepared by a standard method.\(^{25}\) Twenty micrograms of total cellular RNA was denatured with glyoxal, then electrophoresed on a 1.0% agarose gel and blotted onto GeneScreen Plus membranes (NEN Research Products, Boston, MA). The membranes were hybridized with cDNA inserts labeled with \(\alpha\)\(^{32}\)P-dCTP by the random-primer method at 65°C for 14–16 h in a buffer containing 5× SSPE (1× SSPE is 0.18 mol of NaCl, 10 mmol of NaH\(_2\)PO\(_4\)/Na\(_2\)HPO\(_4\) [pH 7.4], and 1 mmol of EDTA), 5× Denhardt’s (1× Denhardt’s is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 1% sodium dodecyl sulfate (SDS), and 10 mg/ml poly(A). The blots were washed with 2× SSC (1× SSC is 0.15 mol of NaCl, 50 mmol of NaH\(_2\)PO\(_4\), and 5 mmol of EDTA), then with 1× SSC containing 1% SDS at room temperature and 65°C. Final washings were with 0.1× SSC containing 0.1% SDS at 65°C. A 2.4-kb full length cDNA designated as EAT/mcl-1 probe was used as a probe.

**RESULTS**

Chemotherapy is widely used in cancer treatment and many agents are known to induce apoptosis through p53-dependent pathways.\(^{26-28}\) Resistance to chemotherapeutic agents has been reported to be acquired by functional mutations of p53 in tumors.\(^{20,30}\) Bcl-2 in certain ovarian cell lines expressing mutant p53 was found to further enhance the levels of resistance to CDDP, one of the most commonly used agents in cancer treatment.\(^{11}\) To determine the role of EAT/mcl-1 gene in resistance to chemotherapeutic agent-induced cell death via p53-independent pathway(s), we transfected EAT/mcl-1 into (10)\(^1\) cells, which do not express p53.\(^{25}\)

**Generation of (10)\(^1\) cells expressing EAT/mcl-1** Cells were transfected with pSR\(\alpha\)-EAT/mcl-1 in addition to pSR\(\alpha\)-neo (denoted as EAT/n). The gene was expressed in 10 of 12 G418-resistant clonal cell populations as assessed by RNA blot hybridization to an EAT/mcl-1...
cDNA probe. Three cell populations expressing EAT/mcl-1 mRNA and protein at different levels (Fig. 1) were chosen for further analysis. Two clonal populations of control G418-resistant cells transfected with pSRα-neo were also chosen.

(10)1 cells and neo/n had no detectable EAT/mcl-1 expression at the mRNA level (Fig. 1A). Each EAT/n clonal population expressed the EAT/mcl-1 gene at different levels; EAT/9 expresses the highest level of EAT/mcl-1 and EAT/7 the lowest, relative to (10)1 rRNA accumulation. Similarly, as evaluated by immunoblot analysis, EAT-transfected cells produced various levels of EAT/mcl-1 (Fig. 1B). The level of EAT/mcl-1 protein was proportional to the transcriptional level found by RNA blot analysis. The exogenously transfected EAT/mcl-1 protein

Fig. 1. Expression of EAT/mcl-1 in (10)1, p53-deficient cells. A. Expression of EAT/mcl-1 gene exogenously transfected in (10)1 cells. Stable transformant (10)1 cells with EAT/mcl-1 were obtained, subcloned and designated as EAT/7, EAT/9, and EAT/14. The neo/6 and neo/21 cells, which were transfected with the neomycin-resistance gene, served as the controls. (10)1 is the parental line and does not express EAT/mcl-1. The position of the specific EAT/mcl-1 transcript is shown by an arrow. General RNA degradation was not observed, since similar amounts of the 28S and 18S rRNAs were recovered from all the cells (bottom). B. Immunoblot analysis of (10)1 cells transfected with the EAT/mcl-1 gene. Sixty micrograms of total protein from each cell line [(10)1, neo/6, neo/21, EAT/7, EAT/9 and EAT/14, respectively, from left to right] was electrophoresed. The blot was probed with rabbit polyclonal antibody to human EAT/mcl-1. The position of EAT/mcl-1 (predicted molecular weight of 37 kilodaltons) is indicated by an arrow on the right. Estimated molecular size is shown in the left.

Fig. 2. Immunohistochemical analysis of (10)1 cells transfected with EAT/mcl-1. (10)1 cells (EAT/9) transfected with EAT/mcl-1 show a positive reaction in their cytoplasm (A), while neo/6 cells do not express the EAT/mcl-1 protein (B).
is localized in the cytoplasm, suggesting that exogenous EAT/mcl-1 may also be localized in the same site (Fig. 2). There was no remarkable morphological difference between the parental cell line and transfected clones as observed by phase contrast microscopy.

**Apoptotic cell death was induced by CDDP in a time-dependent and concentration-dependent manner**

To investigate the response of (10)\(^1\) to chemotherapeutic agents, we first incubated (10)\(^1\) with several concentrations of CDDP and estimated the cell viability at several time points. Cell death of (10)\(^1\) was induced by CDDP in a time-dependent manner, and also in a concentration-dependent manner. As the concentration of the drugs was increased, the cell started to die at an early time-point after treatment. The appearance of dying cells was compatible with the specific features of apoptosis (Fig. 3). The fragmentation of genomic DNA into oligo-nucleosomal-sized units was also detected (Fig. 4).

**Altered resistance of transfected cells to chemotherapeutic agents**

(10)\(^1\), neo\(^n\), and EAT/n clonal populations were tested for relative viability in the presence of several chemotherapeutic agents including CDDP, carboplatin, methotrexate (MTX), etoposide (VEP), mitomycin (MMC), adriamycin (ADM), cyclophosphamide (CPA) and dexamethasone (DEX). EAT/9 and EAT/14 cells showed significant resistance to the toxic effects of CDDP compared with neo/n cells, while EAT/7 cells did not show significant resistance (Fig. 5A). The difference between control cells and EAT/n cells was most distinct with CDDP and carboplatin (Fig. 5B). The cell death of transfected EAT/9 and EAT/14 cells was delayed approximately 48 to 72 h compared with that of neo/n cells. In contrast to CDDP and carboplatin, the sensitivity of EAT/n cells upon exposure to MTX and VEP was similar to that of 10(1) and 10(1)/neo cells; resistance to apoptosis was not detected with these agents (Fig. 5B). Similarly,
EAT/n cells did not exhibit resistance to treatment with MMC, ADM, CPA and DEX (data not shown).

While expression of endogenous EAT/mcl-1 was not detected in (10)1 and (10)1/neo cells, the levels of EAT/mcl-1 transcripts varied in transfected (10)1/EAT cell lines (Fig. 1). Despite this variation, protection (in terms of DNA fragmentation and cell viability) was apparent in EAT/9 and EAT/14 cells. EAT/7 cells without expression of transfected EAT/mcl-1 were indistinguishable from mock-transfected cells. The lack of an expression level-dependent relationship between EAT/mcl-1 transcripts and the ability to delay the onset of apoptosis suggests that these transfectants contained levels of EAT/mcl-1 above the threshold for protection from apoptosis, as previously reported.15)

Since a survival role for EAT/mcl-1 in the regulation of apoptosis has been postulated,4, 15) overexpression of EAT/mcl-1 in (10)1 cells may have increased the ability of those cells to form colonies. If so, EAT/n cells would exhibit increased plating efficiency in comparison with neo/n and (10)1 controls. An association between plating efficiency in the absence of these agents and EAT/mcl-1 expression was not observed (data not shown). Furthermore, the growth rate of cells expressing EAT/mcl-1 was not significantly different from that of the control cells, nor did G418 exposure affect the growth of G418-resistant cells.

A major concern is whether the resistance to CDDP-induced apoptosis as conferred by EAT/mcl-1 allowed the...
cells to survive and then regrow, or whether cell death was merely delayed. We examined the viability of CDDP-treated (10)1 cells at several time points (Fig. 5A). When (10)1 cells were treated with CDDP, EAT/9 and EAT/14 clones were more resistant than the controls. The survival fractions of drug-treated EAT/9 and EAT/14 clones were always higher than those of (10)1 or neo/my, but cell death was apparent at later time points.

**DISCUSSION**

Does EAT/mcl-1 modulate apoptosis via a p53-independent pathway? Considering the sequence similarity between EAT/mcl-1 and Bcl-2,3,32 and the fact that other family members have been reported to influence cell viability, we speculated that EAT/mcl-1 might influence apoptotic pathways and contribute to differentiation through modulation of cell viability. The present studies show that EAT/mcl-1 delayed cell death induced by CDDP and carboplatin in this system. Since drug resistance can be partly attributed to decreased cellular susceptibility to apoptosis,33-38 these data, therefore, support the idea that EAT/mcl-1 contributes to cell survival.

Since (10)1 cells used in this study lack the p53 protein, apoptotic events occur independently of p53. p53 is reported to be involved in the activation of apoptosis, but p53-independent apoptotic pathways also exist.39,40 Although p53 has been shown to induce cell cycle arrest and gene expression (p21/waf-1/cdi-1), the mechanism underlying the p53-independent apoptotic pathway involving EAT/mcl-1 remains unclear.

The inhibition of apoptosis by EAT/mcl-1 may be directly correlated with the mechanism of CDDP-induced cell death.37,41,42 The inhibition of DNA repair by adduction of CDDP to DNA is probably the major mechanism contributing to apoptosis. Following such DNA damage by CDDP, c-abl is involved and then the JNK (SAPK) pathway is activated.43-45 Since EAT/mcl-1 inhibits release of cytochrome c from mitochondria, EAT/mcl-1 may function downstream of c-abl/JNK (SAPK) and therefore inhibit apoptosis induced by CDDP or carboplatin. The other possibility is that EAT/mcl-1 affects the accumulation of CDDP, and thus changes the chemosensitivity.46 EAT/mcl-1 may induce or interact with an efflux pump for these drugs.

**REFERENCES**

1) Hata, J.-I., Fujimoto, J., Ishii, E., Umezawa, A., Kokai, Y., Matsubayashi, Y., Abe, H., Kusakari, S., Kikuchi, H., Yamada, T. and Maruyama, T. Differentiation of human germ cell tumor cells in vivo and in vitro. *Acta Histochem. Cytochem.*, **25**, 563–576 (1992).

2) Maruyama, T., Umezawa, A., Kusakari, S., Kikuchi, H., Nozaki, M. and Hata, J.-I. Heat shock induces differentiation of human embryonal carcinoma cells into trophoblast lineages. *Exp. Cell Res.*, **224**, 123–127 (1996).

3) Umezawa, A., Maruyama, T., Inazawa, J., Imai, S.-I., Takano, T. and Hata, J.-I. Induction of mcl-1/EAT, bcl-2 related gene, by retinoic acid or heat shock in the human
embryonal carcinoma cells, NCR-G3. *Cell Struct. Funct.*, **21**, 143–150 (1996).

4) Kozopas, K. M., Yang, T., Buchan, H. L., Zhou, P. and Craig, R. W. MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. *Proc. Natl. Acad. Sci. USA*, **90**, 3516–3520 (1993).

5) Hockenbery, D., Nunez, G., Milliman, C., Schreiber, R. D. and Korsmeyer, S. J. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature*, **348**, 334–336 (1990).

6) Tsujimoto, Y. Stress-resistance conferred by high level of bcl-2 alpha protein in human B lymphoid cell. *Oncogene*, **4**, 1331–1336 (1989).

7) Siegel, R. J., Bueso, R. C., Cohen, C. and Koss, M. Pulmonary blastoma with germ cell (yolk sac) differentiation: report of two cases. *Mod. Pathol.*, **4**, 566–570 (1991).

8) Bironette, R. P., Echeverri, F., Mahboubi, A. and Green, D. A. Apoptotic cell death induced by c-myc is inhibited by bcl-2. *Nature*, **359**, 552–554 (1992).

9) Boise, L. H., Gonzalez-Garcia, M., Postema, C. E., Ding, L., Linstedt, T., Turkia, L. A., Mao, X., Nunez, G. and Thompson, C. B. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell*, **74**, 597–608 (1993).

10) Oltvai, Z. N., Milliman, C. L. and Korsmeyer, S. J. Bcl-2 heterodimersizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell*, **74**, 609–619 (1993).

11) Yin, X. M., Oltvai, Z. N. and Korsmeyer, S. J. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. *Nature*, **369**, 321–323 (1994).

12) Hengartner, M. O., Ellis, R. E. and Horvitz, H. R. Cae- norhabditis elegans gene ced-9 protects cells from programmed cell death. *Nature*, **356**, 494–499 (1992).

13) Henderson, S., Huen, D., Rowe, M., Dawson, C., Johnson, G. and Richardson, A. Epstein-Barr virus-coded BHRF1 protein, a viral homologue of Bcl-2, protects human B cell from programmed cell death. *Proc. Natl. Acad. Sci. USA*, **90**, 8479–8483 (1993).

14) Tarodi, B., Subramanian, T. and Chinnadurai, G. Epstein-Barr virus BHRF1 protein protects against cell death induced by DNA-damaging agents and heterologous viral infection. *Virology*, **201**, 404–407 (1994).

15) Reynolds, J. E., Yang, T., Quian, L., Jenkinson, J. D., Zhou, P., Eastman, A. and Craig, R. W. McI-1 a member of the Bcl-2 family, delays apoptosis induced by c-myc overexpression in Chinese hamster ovary cells. *Cancer Res.*, **54**, 6348–6352 (1994).

16) Zhou, P., Quian, L., Kozopas, K. M. and Craig, R. W. McI-1, a Bcl-2 family member, delays the death of hematopoietic cells under a variety of apoptosis-inducing conditions. *Blood*, **89**, 630–642 (1997).

17) Reed, C. J. Bcl-2: prevention of apoptosis as a mechanism of drug resistance. *Hematol. Oncol. Clin. North Am.*, **9**, 451–473 (1995).
EAT Inhibits p53-Independent Apoptosis

ptosis and heterodimerization with Bax. *Nature*, **369**, 321–323 (1994).

33) Dive, C. and Hickman, J. Drug-target interactions: only the first step in the commitment to a programmed cell death? *Br. J. Cancer*, **64**, 192–196 (1991).

34) Dive, C. and Wyllie, A. Apoptosis and cancer chemotherapy. In "Cancer Chemotherapy," ed. J. Hickman and T. Tritton, pp. 21–56 (1993). Blackwell Scientific Publications, Oxford.

35) Eastman, A. and Barry, M. Apoptosis as a target for cancer chemotherapy. In "New Molecular Targets for Cancer Chemotherapy," ed. D. Kerr and P. Workman, pp. 143–158 (1994). CRC Press, London.

36) Fritsche, M., Haessler, C. and Brandner, G. Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. *Oncogene*, **8**, 307–318 (1993).

37) Eastman, A. Activation of programmed cell death by anticancer agents: cisplatin as a model system. *Cancer Cells*, **2**, 275–280 (1990).

38) Kondo, S., Barna, P. B., Morimura, T., Takeuchi, J., Yuan, J., Akbasak, A. and Barnett, H. G. Interleukin-1 beta-converting enzyme mediates cisplatin-induced apoptosis in malignant glioma cells. *Cancer Res.*, **55**, 6166–6171 (1995).

39) Fernandez-Sarabia, M. J. and Bischoff, J. R. Bcl-2 associates with the ras-related protein R-ras p23. *Nature*, **366**, 274–275 (1993).

40) Merlo, G. R., Basolo, F., Fiore, L., Duboc, L. and Hynes, N. E. p53-dependent and p53-independent activation of apoptosis in mammary epithelial cells reveals a survival function of EGFR and insulin. *J. Cell Biol.*, **128**, 1185–1196 (1995).

41) Chen, Z., Naito, M., Mashima, T. and Tsuruo, T. Activation of actin-cleavable interleukin 1 beta-converting enzyme (ICE) family protease CPP-32 during chemotherapeutic agent-induced apoptosis in ovarian carcinoma cells. *Cancer Res.*, **56**, 5224–5229 (1996).

42) Zamble, D. and Lippard, S. Cisplatin and DNA repair in cancer chemotherapy. *Trends Biochem. Sci.*, **20**, 435–439 (1995).

43) Seimiya, H., Mashima, T., Toho, M. and Tsuruo, T. c-Jun NH2-terminal kinase-mediated activation of interleukin-1 beta converting enzyme/CED-3-like protease during anticancer drug-induced apoptosis. *J. Biol. Chem.*, **272**, 4631–4636 (1997).

44) Potapova, O., Haghghi, A., Bost, F., Liu, C., Birrer, M., Gjerset, R. and Mercola, D. The Jun kinase/stress-activated protein kinase pathway functions to regulate DNA repair and inhibition of the pathway sensitizes tumor cells to cisplatin. *J. Biol. Chem.*, **272**, 14041–14044 (1997).

45) Kharbanda, S., Ren, R., Pandey, P., Shafman, T., Feller, S., Weichselbaum, R. and Kufe, D. Activation of the c-Abl tyrosine kinase in the stress response to DNA-damaging agents. *Nature*, **376**, 785–788 (1995).

46) Chen, Z. S., Mutoh, M., Sumizawa, T., Furukawa, T., Haraguchi, M., Tani, A., Saijo, N., Kondo, T. and Akiyama, S. An active efflux system for heavy metals in cisplatin-resistant human KB carcinoma cells. *Exp. Cell Res.*, **240**, 312–320 (1998).

47) Strasser, A., Harris, A., Jacks, T. and Cory, S. DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhibited by Bcl-2. *Cell*, **79**, 329–339 (1994).

48) Okamoto-Kubo, S., Nishio, K., Heike, Y., Yoshida, M. and Saijo, N. Apoptosis induced by etoposide in small-cell lung cancer cells. *Cancer Chemother. Pharmacol.*, **33**, 385–390 (1994).

49) Ohmori, T., Podack, E. R., Nishio, K., Takahashi, M., Miyahara, Y., Takeda, Y., Kubota, N., Funayama, Y., Ogasawara, H., Ohira, T., Obta, S. and Saijo, N. Apoptosis of lung cancer cells caused by some anti-cancer agents (MMC, CPT-11, ADM) is inhibited by bcl-2. *Biochem. Biophys. Res. Commun.*, **192**, 30–36 (1993).

50) Tsuruo, T., Hamilton, T. C., Louie, K. G., Behrens, B. C., Young, R. C. and Ozols, R. F. Collateral susceptibility of adriamycin-, melphalan- and cisplatin-resistant human ovarian tumor cells to bleomycin. *Jpn. J. Cancer Res.*, **77**, 941–945 (1986).