0026-895X/02/6206-1400–1408$7.00
MOLECULAR PHARMACOLOGY
Copyright © 2002 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 62:1400–1408, 2002

Vol. 62, No. 6
1770/1024960
Printed in U.S.A.

c-Myc Exerts a Protective Function through Ornithine Decarboxylase against Cellular Insults

JONG KUK PARK, YOUNG MIN CHUNG, SEONGMAN KANG, JAE-UK KIM, YUN-TAIK KIM, HYUNG JUNG KIM, YEUL HONG KIM, JUN SUK KIM, and YOUNG DO YOO

Korea University Cancer Institute (J.K.P., J.S.K., Y.D.Y.), Genomic Research Center for Lung and Breast/Ovarian Cancers (Y.H.K., Y.D.Y.), Brain Korea21 Biomedical Sciences (Y.M.C., J.S.K.), and Department of Internal Medicine (Y.H.K., J.S.K.), Korea University College of Medicine, Seoul, Korea; Department of Life Science, Sogang University, Seoul, Korea (J.K.P., Y.-T.K.); Graduate School of Biotechnology, Korea University, Seoul, Korea (S.K., J.-U.K.); and Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea (H.J.K.)

Received April 2, 2002; accepted September 3, 2002

This article is available online at http://molpharm.aspetjournals.org

ABSTRACT

c-Myc is known to control cell proliferation and apoptosis, and much effort has been focused on elucidating the mechanisms by which c-Myc works. In this study, we show that c-Myc expression is induced by many cellular insults, including cisplatin, doxorubicin, paclitaxel, 5-flourouracil, H_2O_2, and radiation, and the enhanced expression of c-Myc protects against cell death caused by these cellular insults through ornithine decarboxylase (ODC) induction. To investigate the cellular protective role of c-Myc, we constructed a stable transfectant of ODC, one of the many transcriptional targets of c-Myc in cells, and found that enhanced expression of ODC inhibited cell death induced by cellular insults such as cisplatin, H_2O_2, and radiation. We also found that cisplatin activated nuclear factor-κB, and this subsequently induced c-Myc expression, resulting in the blocking of apoptosis through ODC induction. The results herein, therefore, strongly suggest another role for c-Myc in a stress-response function; that is, it promotes cell survival under stressful conditions.

c-Myc is a transcription factor and has a very important role in diverse cellular functions including cell proliferation, transformation, the determination of animal body size, and apoptosis (Schmidt, 1999; Trumpp et al., 2001). Induction of c-Myc is essential for cellular proliferation, and c-Myc controls the cell-cycle machinery. A number of c-Myc target genes such as *cyclin D1*, *cyclin A*, and *eIF4E* are required for cell-cycle progression (Dang, 1999). c-Myc is induced not only by growth factors but also by a variety of apoptotic stimuli, including cytokines, hypoxia, DNA damage, and chemotherapeutic agents (Prendergast, 1999). Therefore, c-Myc has a double-sided effect in terms of its response to anticancer drugs. Although c-Myc induction by some cellular insults has been believed to induce apoptosis, recent reports demonstrate that the inhibition of c-Myc expression enhances CDDP-induced apoptosis rather than blocking apoptosis in vitro and in vivo (Citro et al., 1998; Leonetti et al.,1999).

c-Myc expression was found to be elevated in many tumors, and this is believed to be one cause of cancer formation (Henriksson and Luscher, 1996; Sakamuro and Prendergast, 1999). c-Myc protein is a transcription factor that consists of two major domains. The C-terminal domain, having 90 amino acids, is required for dimerization with Max and for DNA binding activity. The second N-terminal domain has a short, acidic, proline- and glutamine-rich cluster and contributes to the transactivation activity of the Myc protein (Kato et al., 1990; Blackwood and Eisenman, 1991).

Ornithine decarboxylase (ODC) is one of the transcriptional targets of c-Myc. ODC is a key enzyme in polyamine biosynthesis, and it also catalyzes the decarboxylation of ornithine to produce putrescine (Pegg and McCann, 1982; Bello-Fernandez et al., 1993). Intracellular polyamines are essential for cell proliferation and differentiation and are known to have a protective role against cell death caused by various cellular stresses (Rjalakshmi et al., 1978; Brune et al., 1991; Khan et al., 1992). ODC is also believed to be a major target of anticancer drug development and a marker of cancer prognosis (Sharma et al., 1994). In this study, we observed ODC and c-Myc induction, in response to cellular insults. CDDP, 5-FU, paclitaxel, γ-ionizing radiation, and H_2O_2 were used in this study to cause cellular insults. CDDP,

This work was supported by the National Nuclear Research and Development Program from the Ministry of Science and Technology of Korea and the Brain Korea 21 Project in 2002. Work at Yonsei University College of Medicine was supported by grant 2000–2-20800–001-3 from the Basic Research Program of the Korea Science and Engineering Foundation.

ABBREVIATIONS: CDDP, *cis*-diamminedichloroplatinum(II); HDF, human dental fibroblast; RPE, retinal pigment epithelial; NF-κB, nuclear factor-κB; ODC, ornithine decarboxylase; 5-FU, 5-flourouracil; ROS, reactive oxygen species; Gy, gray; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EMSA, electrophoretic mobility shift assay; PI, propidium iodide; JNKDD, dominant-negative c-Jun NH_2-terminal kinase-1 mutant; IκBDD, dominant-negative of IκBα mutant; kbp, kilobase pair.

5-FU, and paclitaxel are effective anticancer agents against many cancers, especially gastric cancers. γ-Ionizing radiation is also a useful cancer-treating modality (Tannock and Hill, 1998). Because several anticancer drugs and diverse cellular stresses are able to induce the generation of reactive oxygen species (ROS), we also used the intracellular oxidant, H_2O_2, to induce stress (Miyajima et al., 1997; Tan et al., 1998; Adler et al., 1999). In the present study, we had two aims: first, to define a possible role for c-Myc in terms of its the cellular effects against various insults, and second, to elucidate the signaling pathway involved in the response to cellular insults.

Materials and Methods

Cell Culture and Anticancer Drugs. Human gastric cancer cells, SNU638 (Yoo et al., 1998), were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated fetal bovine serum (Invitrogen), sodium bicarbonate (2 mg/ml; Invitrogen), penicillin (100 units/ml) and Streptomycin (100 μg/ml; Invitrogen). Human retinal pigment epithelial (RPE) cells and human dental fibroblast (HDF) cells were maintained in Dulbecco's modified Eagle's medium(DMEM; Invitrogen) containing 10% fetal bovine serum, sodium bicarbonate (2 mg/ml), penicillin (100 units/ml) and streptomycin (100 μg/ml) under 5% CO_2 at 37°C. Human RPE cells were kindly provided by Dr. Hum Chung (Seoul National University, Seoul, Korea), and HDF cells were donated by Dr. Young-Sook Son (Korea Cancer Center Hospital, Seoul, Korea). CDDP, 5-FU, paclitaxel, H_2O_2 and all other biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

DNA and Transfection. The *odc* gene in pBlueScript SK^+ was kindly provided by Dr. N. J. Hicock (Thomas Jefferson University, Philadelphia, PA) and the 1.8-kbp *Eco*RI fragment of *odc* was inserted into pUHD10–3 vector, the tetracycline-controlled system. To generate stable cell lines, SNU638 cells (1×10^6) were transferred to a 100-mm tissue-culture dish containing 8 ml of RPMI 1640 medium. After washing with serum-free medium, the cells were incubated with a mixture of 50 μl of Lipofectin (Invitrogen) and 10 μg of DNA in 4 ml of serum-free medium for 8 h. The cells were then cultured in RPMI 1640 medium containing G418 (500 μg/ml; Invitrogen) for 2 to 3 weeks until individual colonies were formed on the plate.

Northern Blot Analysis. Total RNA was isolated with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Total RNA (10 μg per lane) was loaded onto 1% agarose/formaldehyde gel and transferred to a nylon membrane. A ^{32}P-labeled probe was produced using a random primed DNA-labeling kit (Roche, Mannheim, Germany). The blot was probed with a 700-bp ^{32}P-labeled *Eco*RI-*Cla*I fragment of human *odc*, 1.8-kbp *Kpn*I-*Bam*HI fragment of human c-*myc*, and 1-kbp *Eco*RI fragment of human *GAPDH*. All restriction endonucleases were purchased from New England BioLabs (Beverly, MA). Nylon membrane and ^{32}P-dCTP were from NEN PerkinElmer Life Sciences (Boston, MA). The density of each band was quantified using a Fluor-S MultiImager and analyzed with Quantity One software (Bio-Rad, Hercules, CA).

Immunoblot Analysis. Immunoblot analysis was performed as described previously (Yoo et al., 1998). The visualization of the immunoreactivities of anti-cyclin D1, cyclin A, and IκB-α antibodies were performed by using enhanced chemiluminescence. All antibodies except for β-actin antibody (Sigma) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and the enhanced chemiluminescence kit was from Amersham Biosciences (Little Chalfont, Buckinghamshire, UK). The density of each band was quantified using a Fluor-S MultiImager and analyzed with Quantity One software (Bio-Rad).

DNA Fragmentation Assay. Cells were grown until they were ~80% confluent, then they were exposed to trypsin and replated on tissue-culture flasks at 10,000 cells/cm^2. Cells were then treated with CDDP. After being cultured for 48 h, the cells were harvested and washed once with phosphate-buffered saline. The pellet was then resuspended in lysis buffer (10 μM Tris-HCl, pH 8.0, 75 mM NaCl, 10 mM EDTA, 0.5% SDS) containing proteinase K (10 mg/ml), and the suspension was incubated for 3 h at 50°C with gentle shaking. DNA was isolated from the cell lysates with phenol/chloroform/isoamyl alcohol (25:24:1) and precipitated with isopropanol. The pellet was washed once with 75% ethanol, dissolved in 10 mM Tris, pH 7.5, and 0.1 mM EDTA containing 150 μg/ml RNase, and incubated for 3 h at 50°C. The DNA samples so obtained were analyzed for DNA fragmentation by electrophoresis on a 1.5% agarose gel.

Clonogenic Assay. SNU638/neo, ODC-overexpressing cells were seeded in triplicate at 300 cells per 100-mm tissue-culture dishes. The cells were then incubated for 24 h in a CO_2 incubator at 37°C and

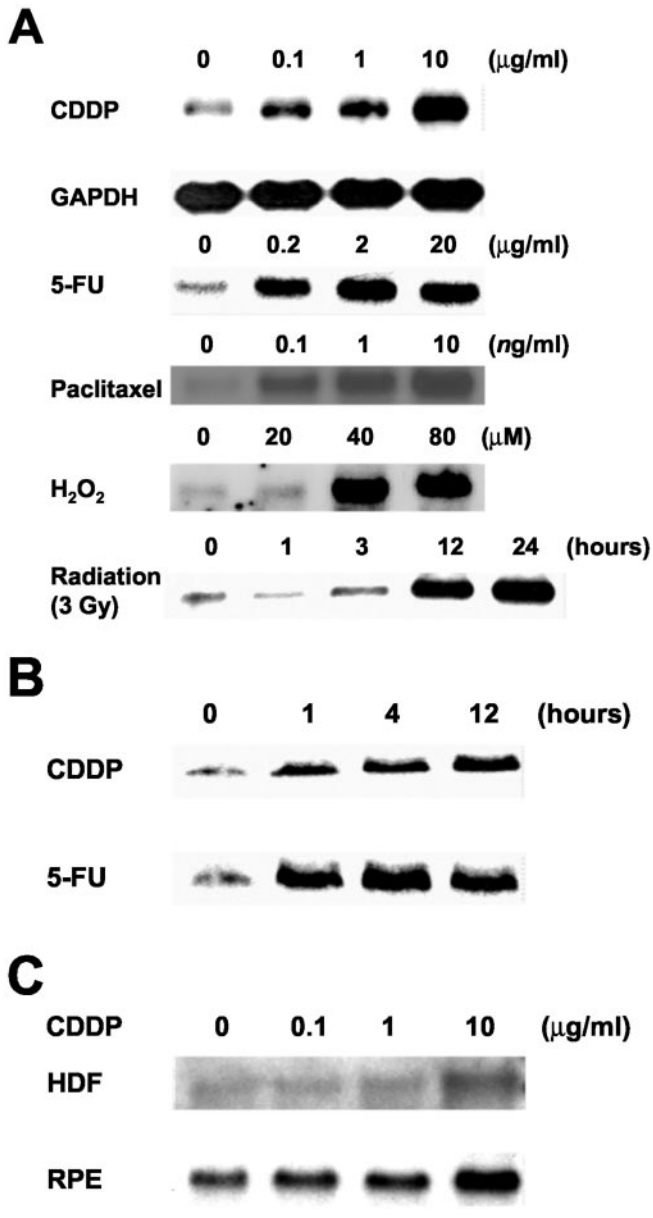


Fig. 1. A, Northern blot analysis of c-Myc in the SNU638 cell line. The cells were treated with various concentration of CDDP, 5-FU, paclitaxel, and H_2O_2 and then harvested at 24 h. Irradiated cells were harvested at 1, 3, 12, and 24 h after 3 Gy of ionizing γ-irradiation. Northern blotting was performed as described under *Materials and Methods*. B, immunoblot analysis of c-Myc. SNU638 cells were treated with CDDP (10 μg/ml) or 5-FU (5 μg/ml) and harvested 1, 4, or 12 h later. C, HDF and RPE cells were treated with various concentrations of CDDP, harvested at 24 h, and the analyzed via Northern blot for c-Myc.

irradiated with γ-rays from a ^{137}Cs γ-ray source at dosages of 1, 3, and 6 Gy. Colonies were allowed to grow for 10 days and stained with 1% methylene blue in methanol. Colonies larger than 200 μm in diameter were counted by using a colony counter. The ^{137}Cs γ-ray source was obtained from Atomic Energy of Canada, Ltd. (Ontario, Canada). The colony counter was obtained from Imaging Products (Chantilly, VA).

Extraction of Nuclear Protein. Drug-treated cells were harvested, and pellets were washed with 0.4 ml of buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride). Cells were then suspended in 0.4 ml of buffer A containing 0.1% Nonidet P-40, and the suspension was incubated on ice for 15 min. Cytoplasmic protein was removed by centrifugation in a microcentrifuge at 12,000 rpm for 30 s at 4°C, and the pellet was resuspended in 0.1 ml of buffer B (20 mM HEPES, pH 7.9, 20% glycerol, 0.42 M NaCl, 10 mM KCl, 1 mM EDTA, 0.5 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride). Cellular debris was removed by centrifugation in a microcentrifuge at 12,000 rpm for 15 min at 4°C.

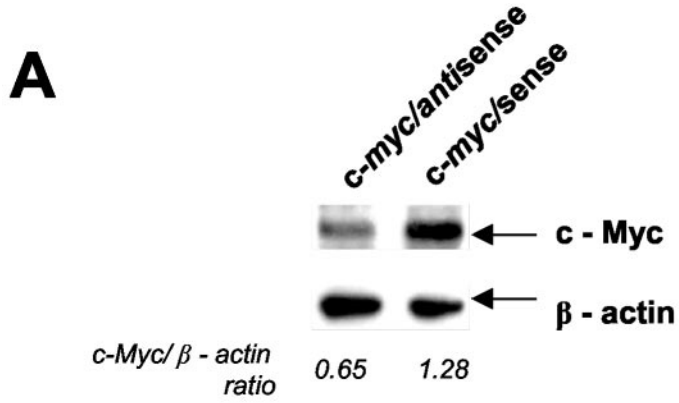
Electrophoretic Mobility Shift Assay. EMSA kit was purchased from Promega (Madison, WI). After cells had been treated with CDDP, EMSA was performed according to the manufacturer's instructions. DNA binding of NF-κB was detected by using a ^{32}P-labeled double-stranded consensus oligonucleotide (5′-AGTTGAGGGGACTTTCCCAGGC-3′). To detect supershift, anti-NF-κB (p65) antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was pretreated into the reaction mixture.

Cell-Cycle Analysis. After drug treatment, both detached and attached cells were harvested at 2500 rpm for 5 min. The cells were washed with phosphate-buffered saline once and fixed with 85% ethanol at 4°C and then treated with 50 μg/ml of propidium iodide (PI) and 100 μg/ml of RNase A for 30 min at 37°C. Cell-cycle analysis was performed by FACScan flow cytometry (BD Biosciences, San Jose, CA).

Statistical Analysis. Statistical analysis for significant differences between the control and experimental groups were evaluated using Student's *t* test. Means, S.E., and *P* values were calculated using Prism for Windows (GraphPad Software, San Diego, CA).

Results

Increased Expression of c-Myc Induced by Cellular Insult Is Associated with Cell Survival. Various cellular insults, including CDDP, 5-FU, paclitaxel, γ-ionizing radiation, and H_2O_2, induced c-Myc expression (Fig. 1, A and B) in



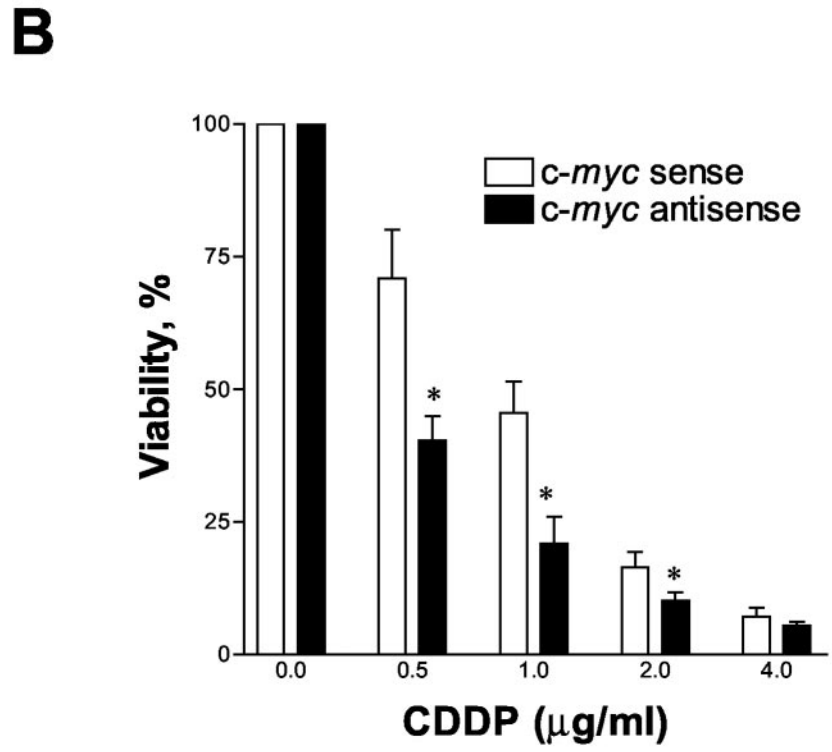


Fig. 2. Sense (5′-AAGCATACGGGGTGT-3′) or antisense (5′-AACGTTGAGGGGCAT-3′) synthetic oligonucleotides (10 μM) of c-*myc* were transiently transfected twice into SNU638 cells. A, the inhibition of c-Myc expression by its antisense oligonucleotide was analyzed by immunoblotting. β-actin was loaded as a control. B, the cells were then treated with 0.5, 1, 2, or 4 μg/ml of CDDP for 72 h. Surviving cells were counted under the microscope. This experiment was performed in triplicate and was repeated three times. Results shown are the mean percentages (±S.E.) of three experiments; *, $P < 0.05$.

SNU638, a gastric cancer cell line. The enhanced expression of c-Myc was detected in a dose-dependent manner against 0.1, 1, and 10 μg/ml of CDDP; 0.2, 2, and 20 μg/ml of 5-FU; 0.1, 1, and 10 ng/ml of paclitaxel; 20, 40, and 80 μM of H_2O_2, and in a time-dependent manner against 10 μg/ml of CDDP, 5 μg/ml of 5-FU, and 3 Gy of γ-radiation. In addition, this induction was also observed in healthy human cells including human RPE cells and HDF cells (Fig. 1C). These results indicate the ubiquitous nature of c-Myc induction by cellular stresses in human cells. The role of c-Myc in SNU638 cells was examined using synthetic sense and antisense oligonucleotides of c-*myc*. A previous study showed that treatment of some cancer cells with c-*myc* antisense oligonucleotide made the cells more sensitive to CDDP (Leonetti et al., 1999). Therefore, we examined whether c-Myc antisense enhanced CDDP-induced cell death in SNU638 cells. The cells were transiently transfected with 10 μM of sense or antisense c-*myc* oligonucleotides, and then treated with 0.5, 1, 2, or 4 μg of CDDP for 72 h. c-Myc expression was inhibited by treatment with the *c-myc* antisense oligonucleotide (Fig. 2A).

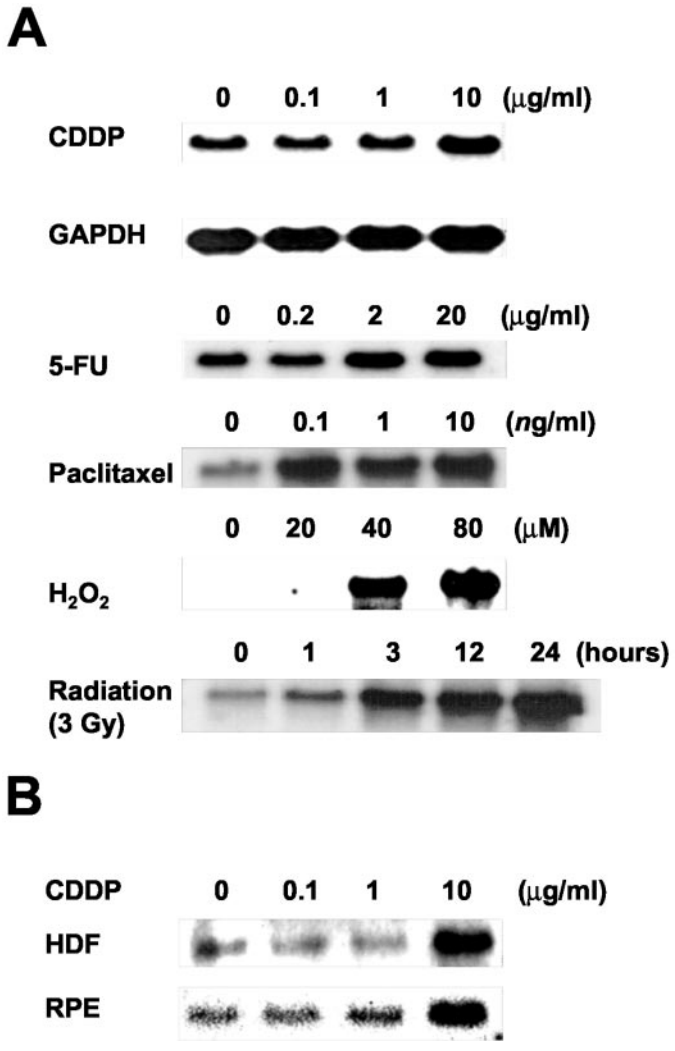


Fig. 3. A, SNU638 cells were treated with various concentrations of CDDP, 5-FU, paclitaxel, and H_2O_2. Northern blot analysis was then performed for ODC. Irradiated cells were harvested at 1, 3, 12, or 24 h after 3 Gy of γ-irradiation. B, HDF and RPE cells were treated with various concentrations of CDDP and harvested at 24 h. Northern blotting was then performed for ODC.

As shown in Fig. 2B, the inhibition of c-*myc* expression by its antisense oligonucleotide enhanced CDDP-induced cell death. These results suggest that the increased expression of c-Myc might protect SNU638 cells from stresses rather than from apoptosis.

ODC Was also Induced against Various Cellular Stresses and This Induction Was Regulated by c-Myc. Because ODC is one of the many transcriptional targets of c-Myc, we investigated whether the protective role of c-Myc is related to ODC induction. As shown in Fig. 3A, various cellular insults such as 0.1, 1, and 10 μg/ml of CDDP; 0.2, 2, and 20 μg/ml of 5-FU; 0.1, 1, and 10 ng/ml of paclitaxel; 20, 40, and 80 μM of H_2O_2; and 3Gy of γ-radiation induced ODC expression as well as c-Myc. This induction was also observed in healthy human cells, such as RPE and HDF cells (Fig. 3B). To investigate whether CDDP-induced ODC expression occurred through c-Myc induction, the cells were transiently transfected with 10 μM of sense or antisense synthetic c-*myc* oligonucleotides and then administered 0.1 or 1 μg/ml of CDDP. As shown in Fig. 4A, ODC induction by CDDP treatment was blocked in antisense oligonucleotide-transfected cells, suggesting that ODC was induced by CDDP through c-Myc induction. Cyclin D1 and cyclin A are also known transcription targets of c-Myc. Therefore, to investigate whether CDDP-induced c-Myc induction controls the expression of cell-cycle positive regulators, immunoblot analyses were carried out for cyclin D1 and cyclin A in CDDP-treated cells. When the cells were treated with CDDP, the expression of cyclin D1 and cyclin A was not induced for up to 24 h (Fig. 4B), leading us to conclude that c-Myc induction by CDDP treatment is not related to cell proliferation. To identify a cell-protective role of ODC, we constructed a stable transfectant of ODC, which was examined by Northern blot analysis (Fig. 5A). The enhanced expression of ODC prevented cell death induced by CDDP, H_2O_2, and γ-ionizing radiation, as shown in Fig. 5, B through D. In the case of γ-ionizing radiation, ODC induction protected from cell death only at low doses of radiation. A DNA fragmentation assay showed

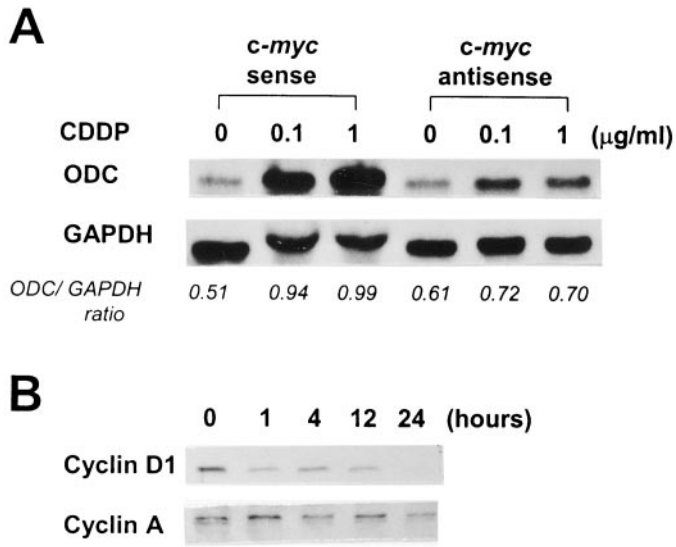


Fig. 4. A, SNU638 cells were transiently transfected with 10 μM of sense and antisense synthetic oligonucleotides of c-*myc* and then treated with CDDP for 24 h before Northern blotting. B, SNU638 cells were treated with CDDP (10 μg/ml) and harvested at 0, 1, 4, 12, and 24 h for immunoblot analysis.

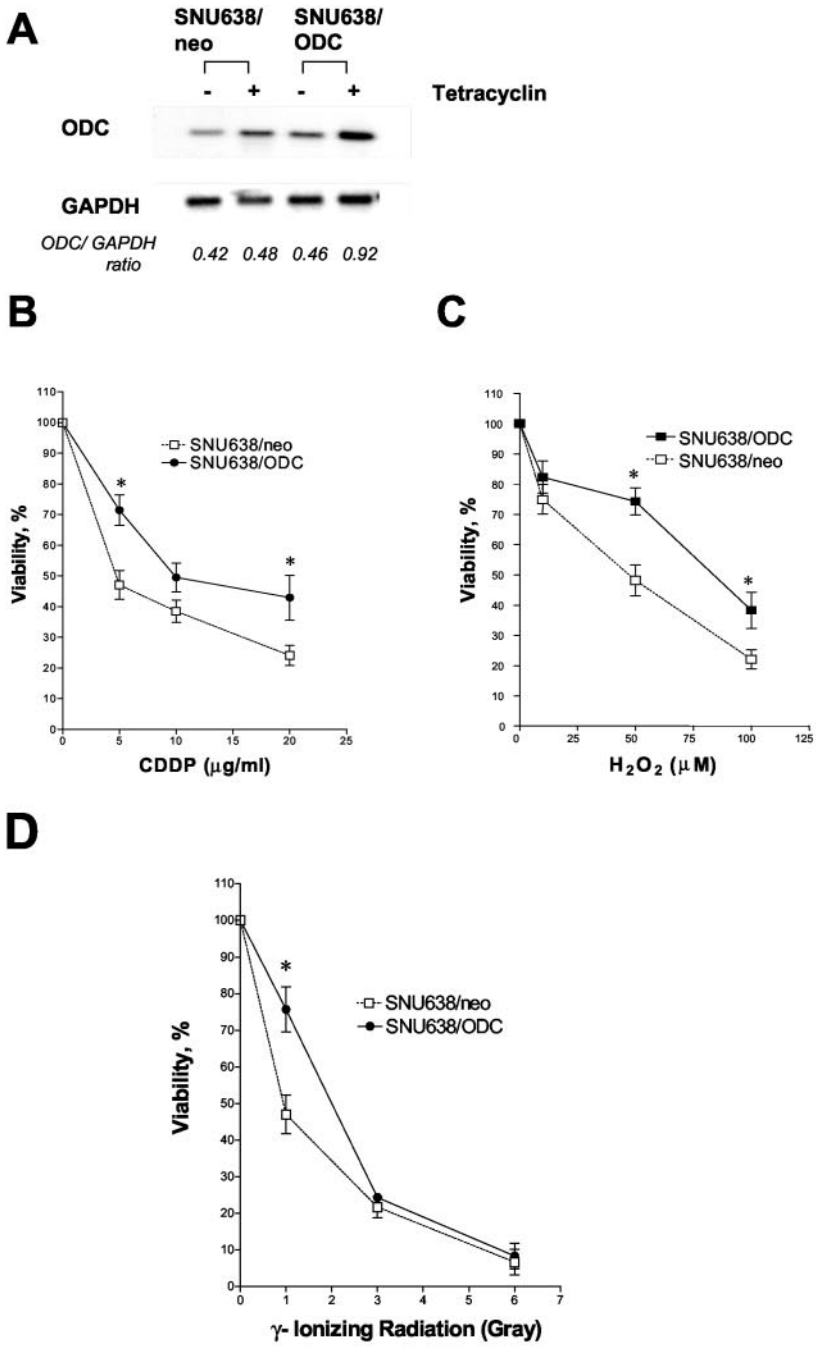


Fig. 5. SNU638/neo and SNU638/ODC (ODC-overexpressing cells) were pretreated with 1 μg/ml of tetracycline for 1 h before each experiment. A, construction of ODC-overexpressing cells. SNU638 cells were transfected with the *odc* gene, and ODC expression was examined by Northern blot analysis with or without 10 μg/ml of tetracycline. Bottom, GAPDH. B, CDDP-induced cell death. Cells were treated with 5, 10, 15, and 20 μg/ml of CDDP for 24 h, and the surviving cells were counted under the microscope. C, H_2O_2-induced cell death. Cells were treated with 10, 50, and 100 μM of H_2O_2 for 24 h, and surviving cells were counted under the microscope. D, radiation-induced cell death. Cells were irradiated with 1, 3, or 6 Gy of ionizing γ-radiation and a clonogenic assay was performed. Results shown in B, C, and D are the mean percentages ($\pm$ S.E.) of three independent experiments; *, $P < 0.05$. E, DNA fragmentation assay. Cells were treated with 2, 5, or 10 μg/ml of CDDP for 48 h. Total DNA was loaded onto 1% agarose gel and visualized by use of ethidium bromide staining.

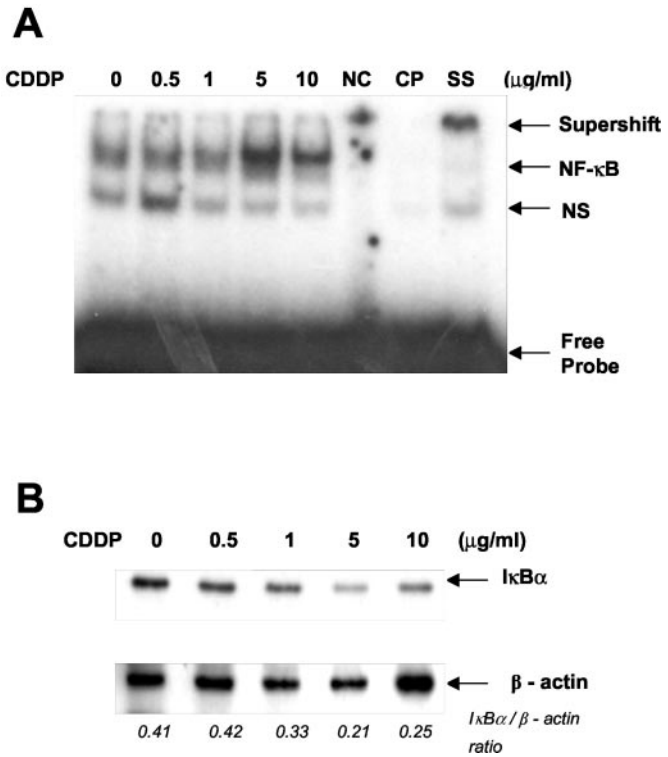


Fig. 6. SNU638 cells were treated with 0, 0.5, 1, 5, or 10 μg/ml of CDDP for 24 h. A, NF-κB DNA-binding activity was determined by EMSA. NC, negative control: no protein was added to reaction mixture. CP, cold probe: 0.35 pmol of no-labeled probe was added. NS, nonspecific band; SS, supershift. Anti-NF-κB (p65 fragment) was added to the reaction mixture before the ^{32}P-labeled probe. B, immunoblot analysis for IκBα and β-actin were performed on the whole extract. β-Actin was used as a control.

that the progression of apoptosis was also inhibited by the enhanced expression of ODC (Fig. 5E). Therefore, we postulate that the protective function of c-Myc is the result of ODC induction.

CDDP Induced c-Myc Expression through NF-κB Activation. Because NF-κB is activated by many cellular stresses and is a known activator of c-Myc (Mercurio and Manning, 1999), we examined whether CDDP induced NF-κB activation in SNU638 cells. The increased DNA-binding activity of NF-κB and reduced IκBα expression were observed after treating cells with CDDP in a dose-dependent manner (Fig. 6). When cells were treated with 10 μg/ml of CDDP, less NF-κB activation and IκBα reduction were observed compared with cells treated with 5 μg/ml of CDDP. To further confirm that NF-κB activation induced c-Myc expression, we constructed a stable transfectant overexpressing a dominant-negative of IκBα mutant (IκBDD) (Fig. 7A), in which serine 32 and serine 36 were substituted by alanine, and this was found to result in the inhibition of NF-κB activation. We also used *neo*^{r}-transfectant and the dominant-negative c-Jun NH_2-terminal kinase-1 mutant (JNKDD)-overexpressing cells as a control. The dominant-negative c-Jun NH_2-terminal kinase-1 mutant contained alanine and phenylalanine instead of threonine 183 and tyrosine 185 (Gupta et al., 1995). As shown in Fig. 7B, CDDP treatment did not induce c-Myc expression in IκBDD-overexpressing cells; however, CDDP treatment effectively induced c-Myc expression in *neo*^{r}-transfectant cells in which c-Myc expression in JNKDD-overexpressing cells was less induced than that of *neo*^{r}-transfectant cells. To verify that IκBDD inhibits its target, EMSA and immunoblot analysis was performed. Treatment with 10 μg/ml of CDDP induced NF-κB activation in *neo*^{r}-transfectant cells and JNKDD-overexpressing cells. However, the inhibition of NF-κB activation was observed in IκBDD-overexpressing cells (Fig, 7C). This result was also confirmed by immunoblot analysis, in which IκBα reduction was inhibited (Fig. 7D). This result indicates that CDDP treatment induced c-Myc expression through NF-κB activation. We showed previously that the inhibition of c-Myc expression by antisense *c-myc* oligonucleotide enhanced CDDP-induced cell death in SNU638 cell line (Fig. 2). Because the enhanced expression of IκBDD also inhibited c-Myc induction, we next examined whether the overexpression of IκBDD sensitized CDDP-induced cell death. Thus, we treated three cell lines with various concentrations of CDDP for 72 h and measured CDDP-induced cell death by cell counting and PI staining. It was found that CDDP-induced cell death was enhanced in IκBDD-overexpressing cells (Fig. 8, A and B).

Discussion

c-Myc expression is rapidly induced by mitogenic stimulation and remains elevated during cell-cycle progression (Schmidt, 1999), and it is also induced by cell-death signaling, resulting in apoptosis (Dang, 1999). In the present study, we defined the role of c-Myc in gastric cancer cells and observed c-Myc induction by various cellular insults. Therefore, we investigated whether c-Myc induction was involved in apoptosis or had some other function. When SNU638 cells were subjected to various stresses, the induction of c-Myc was observed in a time- and dose-dependent manner. However, this induction did not seem to be involved in apoptosis, because antisense c-Myc sequence sensitized CDDP-induced cell death in SNU638 cells. This result suggested that the increased expression of c-Myc plays a role in protecting cells from death rather than one of inducing apoptosis. This fact was also confirmed by the expression patterns of cyclin D1 and cyclin A, which are downstream regulators of c-Myc–mediated cell proliferation (Dang, 1999). Thus, the present study strongly indicates that c-Myc has a role in protecting cells from death during periods of cellular stress. Because c-Myc seemed to use a signaling pathway other than the proliferative pathway, we attempted to identify this pathway. ODC is a downstream signaling mediator of c-Myc and also protects cells from cellular insults. In the present study, many cellular insults that induced c-Myc expression were also found to enhance ODC expression, without inducing the target genes required for cell-cycle progression. These inductions of c-Myc and ODC seemed to be mediated by the production of ROS, which were produced by CDDP and γ-radiation, and because H_2O_2 also induced c-Myc and ODC expression (Miyajima et al., 1997; Tan et al., 1998; Adler et al., 1999).

The protective role of ODC in cells seems to be associated with the production of spermidine, one of the products of the polyamine biosynthetic pathway (Pegg and McCann, 1982). It is known that polyamines and their analogs have a protective role from DNA strand breakage induced by ROS and apoptosis (Brune et al., 1991; Ha et al., 1998; Douki et al., 2000). We observed that spermidine treatment inhibited CDDP-induced cell death in a dose-dependent manner.

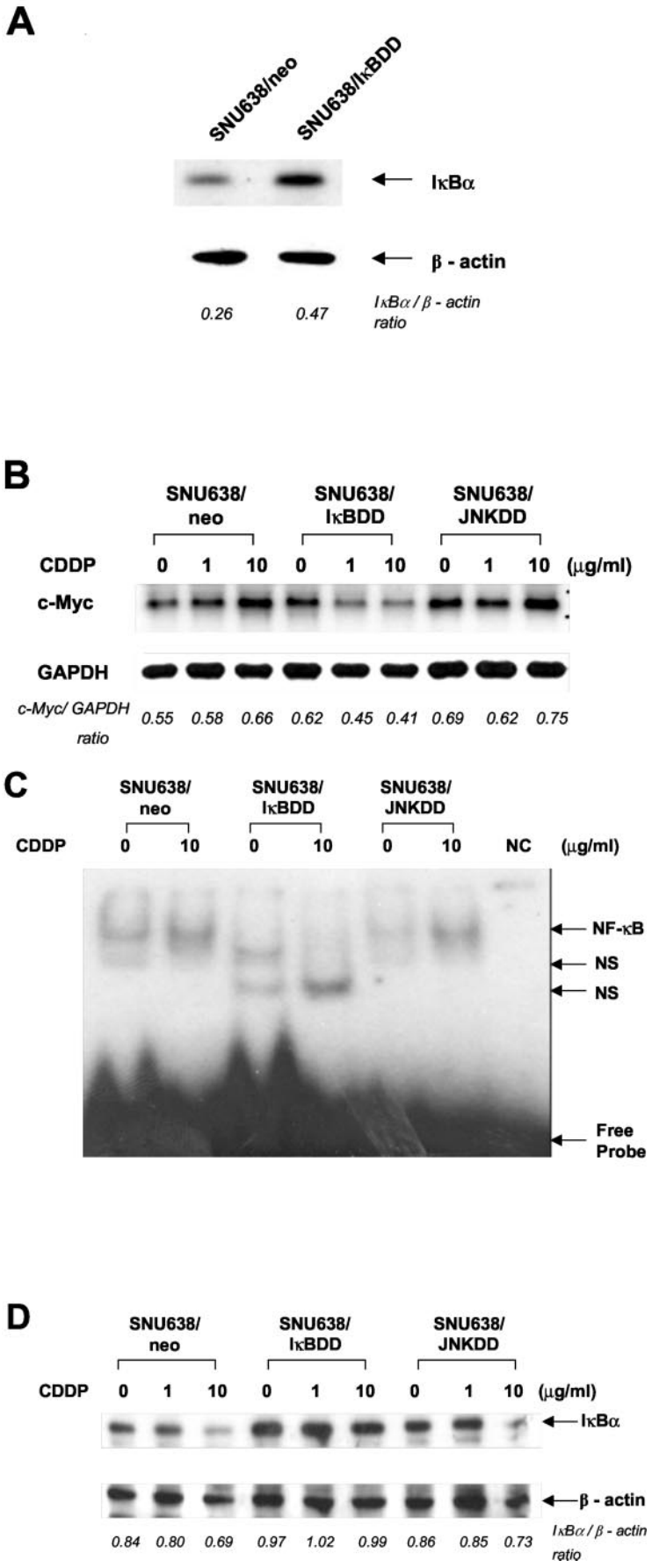


Fig. 7. A, construction of dominant-negative IκBDD-overexpressing cells was confirmed. The overexpression of IκBDD was examined by immunoblot analysis. Lower bands are β-actin. B, blocking of c-*myc* expression by IκBDD. Cells were treated with 1 or 10 μg/ml of CDDP, and Northern blotting was performed. C, EMSA was performed with *neo*^{r}-transfectant cells, JNKDD-overexpressing cells, and IκBDD-overexpressing cells exposed to 10 μg/ml of CDDP for 24 h. NC, negative control; NS, nonspecific band. D, immunoblot assay was done with IκBα and β-actin antibody. Cells were treated with 0, 1, or 10 mg/ml of CDDP for 24 h, and whole-protein fractions of these cells were extracted for immunoblot assay.

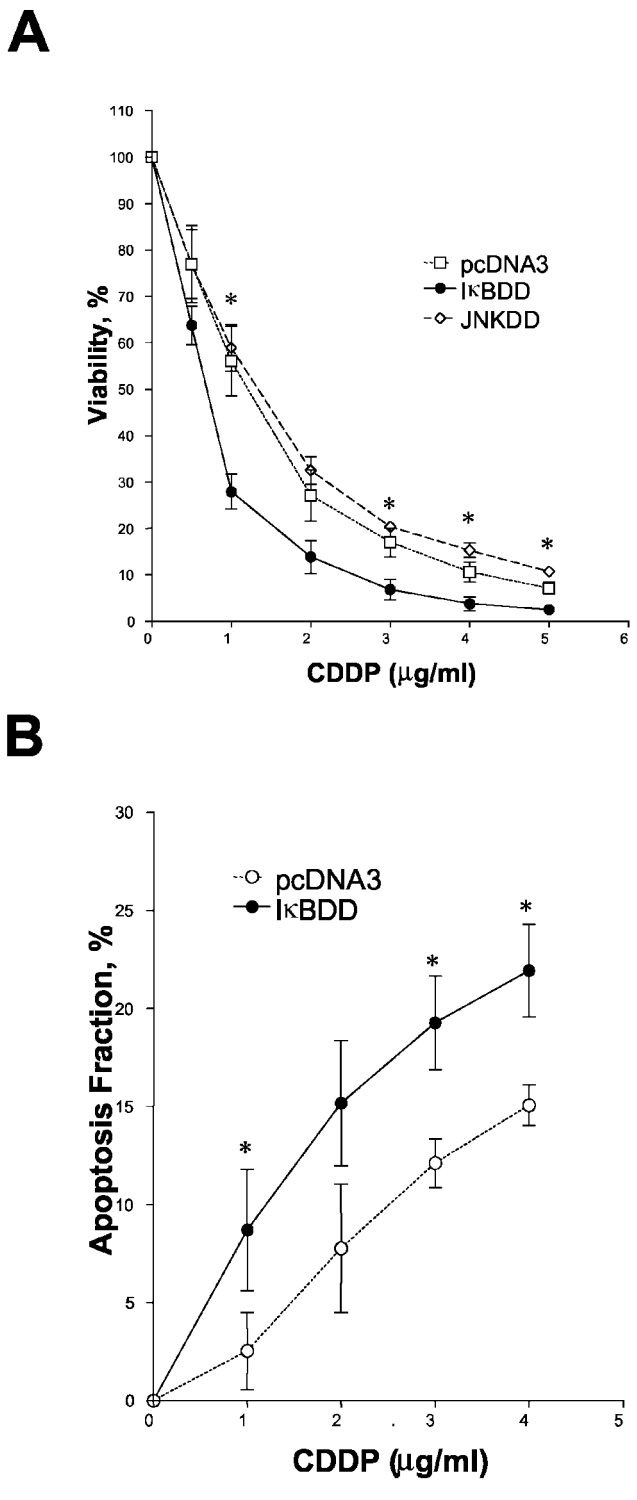


Fig. 8. IκBDD enhanced CDDP-induced cell death. A, for the cell-count assay, 5×10^4 cells of each transfectant were seeded into 6-well plates, treated with 0.5, 1, 2, or 4 μg/ml of CDDP for 72 h, and counted. Results shown are the mean percentages (± S.E.) of three independent experiments; *, $P < 0.05$. B, apoptosis rate determination. Cells (10^6) were seeded into a 100-mm cell-culture dish and then treated with 1, 2, 3, or 4 μg/ml of CDDP for 72 h. Detached and attached cells were stained with PI. Cell-cycle distribution was performed using FACScan flow cytometry, in triplicate. Results are expressed as the mean percentages (± S.E.) of three independent experiments; *, $P < 0.05$.

Therefore, it is highly likely that the enhanced production of spermidine by ODC induction protected DNA from DNA-damaging agents such as CDDP. This phenomenon might contribute to cancer progression, because several studies have detected ODC overexpression in some tumors and have associated this with a poor prognosis (O'Brien et al., 1997; Mohan et al., 1999) and with neoplastic growth in vitro and in vivo (Pegg, 1988).

Our study also provides some information about the signal pathway between NF-κB and c-Myc. NF-κB is a central regulator of stress response and is activated by a variety of stimuli, especially γ-radiation, oxidative stress, and chemotherapeutic agents, including CDDP and paclitaxel (Pahl, 1999). NF-κB has a dual function, i.e., it shows antiapoptotic activity in tumor necrosis factor-α–induced apoptosis and pro-apoptotic activity in p53-induced apoptosis (Ryan et al., 2000). NF-κB, in this study, responded to CDDP, and this resulted in the inhibition of CDDP-induced apoptosis. We also observed that blocking NF-κB enhanced CDDP-induced cell death in IκBDD-overexpressing cells, which implied that CDDP induced the activation of c-Myc through NF-κB in these cells. This result was also in accord with published results, which show that the suppression of NF-κB activation enhanced cell death in vitro caused by challenge with chemotherapeutic agents (Baldwin, 2001). NF-κB seemed to play a role as an important transcription factor by coupling stress-responsiveness and the antiapoptotic pathway. In the present study, it was found that c-Myc has a protective role against the apoptotic effect of cellular insults. Moreover, our study lead us to the conclusion that c-Myc is located downstream of and is under the control of NF-κB in gastric cancer cells. The induction of c-Myc by CDDP was followed by increased ODC expression, which resulted in the protection of the cells from CDDP cytotoxicity. From these results, we also suggest that c-Myc, ODC, and NF-κB may be important targets of combination therapies for enhancing the efficacy of anticancer drugs and for gastric cancer drug development.

References

Adler V, Yin Z, Tew KD, and Ronai Z (1999) Role of redox potential and oxygen species in stress signaling. *Oncogene* **18:**6104–6111.

Baldwin AS Jr (2001) The transcription factor NF-κB and human disease. *J Clin Invest* **107:**241–246.

Bello-Fernandez C, Packham G, and Cleveland JL (1993) The ornithine decarboxylase gene is a transcriptional target of c-Myc. *Proc Natl Acad Sci USA* **90:**7804–7808.

Blackwood EM and Eisenman RN (1991) Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science (Wash DC)* **251:**1211–1217.

Brune B, Hartzell P, Nicotera P, and Orrenius S (1991) Spermidine prevents endonuclease activation and apoptosis in thymocytes. *Exp Cell Res* **195:**323–329.

Citro G, D'Agnano I, Leonetti C, Perini R, Bucci B, Zon G, Calabretta B, and Zupi G (1998) c-Myc antisense oligodeoxynucleotides enhance the efficacy of CDDP in melanoma chemotherapy *in vitro* and in nude mice. *Cancer Res* **58:**283–289.

Dang CV (1999) c-Myc target genes involved in cell growth, apoptosis and metabolism. *Mol Cell Biol* **19:**1–11.

Douki T, Bretonniere Y, and Cadet J (2000) Protection against radiation-induced degradation of DNA bases by polyamines. *Radiat Res* **153:**29–35.

Gupta S, Campbell D, Derijard B, and Davis RJ (1995) Transcription factor ATF2 regulation by the JNK signal transduction pathway. *Science (Wash DC)* **267:**389–393.

Ha HC, Yager JD, Woster PA, and Casero RA Jr (1998) Structural specificity of polyamines and polyamine analogues in the protection of DNA from strand breaks induced by reactive oxygen species. *Biochem Biophys Res Commun* **244:**298–303.

Henriksson M and Luscher B (1996) Proteins of the Myc network: essential regulators of cell growth and differentiation. *Adv Cancer Res* **68:**109–182.

Kato GJ, Barrett J, Villa-Garcia M, and Dang CV (1990) An amino-terminal c-*myc* domain required for neoplastic transformation activates transcription. *Mol Cell Biol* **10:**5914–5920.

Khan AU, Di Mascio P, Medeiros MH, and Wilson T (1992) Spermine and spermidine protection of plasmid DNA against single-strand breaks induced by singlet oxygen. *Proc Natl Acad Sci USA* **89:**11428–11430.

Leonetti C, Biroccio A, Candiloro A, Citro G, Fornari C, Mottolese M, Del Bufalo D,

and Zupi G (1999) Increase of CDDP sensitivity by c-Myc antisense oligodeoxynucleotides in a human metastatic melanoma inherently resistant to CDDP. *Clin Cancer Res* **5:**2588–2595.
Mercurio F and Manning AM (1999) NF-κB as a primary regulator of the stress response. *Oncogene* **18:**6163–6171.
Miyajima A, Nakashima J, Yoshioka K, Tachibana M, Tazaki H, and Murai M (1997) Role of reactive oxygen species in cis-dichlorodiammineplatinum-inducing cytotoxicity on bladder cancer cells. *Br J Cancer* **76:**206–210.
Mohan RR, Challa A, Gupta S, Bostwick DG, Ahmad N, Agarwal R, Marengo SR, Amini SB, Paras F, MacLennan GT, et al. (1999) Overexpression of ornithine decarboxylase in prostate cancer and prostatic fluid in humans. *Clin Cancer Res* **5:**143–147.
O'Brien TG, Megosh LC, Gilliard G, and Soler AP (1997) Ornithine decarboxylase overexpression is a sufficient condition for tumor promotion in mouse skin. *Cancer Res* **57:**2630–2637.
Pahl HL (1999) Activators and target genes of Rel/NF-κB transcription factors. *Oncogene* **18:**6853–6866.
Pegg AE (1988) Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res* **48:**759–774.
Pegg AE and McCann PP (1982) Polyamine metabolism and function. *Am J Physiol* **243:**C212–C221.
Prendergast GC (1999) Mechanisms of apoptosis by c-Myc. *Oncogene* **18:**2967–2987.
Rjalakshmi S, Rao PM, and Sarma DS (1978) Modulation of carcinogen chromatin-DNA interaction by polyamines. *Biochem Biophys Res Commun* **81:**936–940.
Ryan KM, Ernst MK, Rice NR, and Vousden KH (2000) Role of NF-κB in p53-mediated programmed cell death. *Nature (Lond)* **404:**892–897.
Sakamuro D and Prendergast GC (1999) New Myc-interacting proteins: a second Myc network emerges. *Oncogene* **18:**2942–2954.
Schmidt EV (1999) The role of c-Myc in cellular growth control. *Oncogene* **18:**2988–2996.
Sharma S, Stutzman JD, Kelloff GJ, and Steele VE (1994) Screening of potential chemopreventive agents using biochemical markers of carcinogenesis. *Cancer Res* **54:**5848–5855.
Tan S, Sagara Y, Liu Y, Maher P, and Schubert D (1998) The regulation of reactive oxygen species production during programmed cell death. *J Cell Biol* **141:**1423–1432.
Tannock IF and Hill RP (1998) *The Basic Science of Oncology.*3rd ed. McGraw-Hill, New York.
Trumpp A, Refaeli Y, Oskarsson T, Gasser S, Murphy M, Martin GR, and Bishop JM (2001) c-Myc regulates mammalian body size by controlling cell number but not cell size. *Nature (Lond)* **414:**768–773.
Yoo YD, Park JK, Choi J-Y, Lee K-H, Kang Y-K, Kim CS, Shin SW, Kim YH, and Kim JS (1998) CDK4 down-regulation induced by paclitaxel is associated with G_1 arrest in gastric cancer cells. *Clin Cancer Res* **4:**3063–3068.

Address correspondence to: Dr. Young Do Yoo, Genomic Research Center for Lung and Breast/Ovarian Cancers, Korea University Cancer Institute, Korea University College of Medicine, Anam Hospital, 126–1, 5ka, Anam-dong, Sungbuk-ku, Seoul 136-705, Korea. E-mail: ydy@kumc.or.kr