Inhibition of Extracellular Signal-regulated Protein Kinase or c-Jun N-terminal Protein Kinase Cascade, Differentially Activated by Cisplatin, Sensitizes Human Ovarian Cancer Cell Line*

We have studied the roles of c-Jun N-terminal protein kinase (JNK) and extracellular signal-regulated protein kinase (ERK) cascade in both the cisplatin-resistant Caov-3 and the cisplatin-sensitive A2780 human ovarian cancer cell lines. Treatment of both cells with cisplatin but not transplatin isomer activates JNK and ERK. Activation of JNK by cisplatin occurred at 30 min, reached a plateau at 3 h, and declined thereafter, whereas activation of ERK by cisplatin showed a biphasic pattern, indicating the different time frame. Activation of JNK by cisplatin was maximal at 1000 μM, whereas activation of ERK was maximal at 100 μM and was less at higher concentrations, indicating the different dose dependence. Cisplatin-induced JNK activation was neither extracellular and intracellular Ca\(^{2+}\)- nor protein kinase C-dependent, whereas cisplatin-induced ERK activation was extracellular and intracellular Ca\(^{2+}\)-dependent and protein kinase C-dependent. A mitogen-activated protein kinase/extracellular signal-regulated protein kinase inhibitor, PD98059, had no effect on the cisplatin-induced JNK activity, suggesting an absence of cross-talk between the ERK and JNK cascades. We further examined the effect of each cascade on the viability following cisplatin treatment. Either exogenous expression of dominant negative c-Jun or the treatment by PD98059 induced sensitivity to cisplatin in both cells. Our findings suggest that cisplatin-induced DNA damage differentially activates JNK and ERK cascades and that inhibition of either of these cascades sensitizes ovarian cancer cells to cisplatin.

Various cellular stimuli that control cell growth and differentiation cause a rapid increase in the enzymatic activity of a family of serine/threonine kinases known as the mitogen-activated protein (MAP)\(^1\) kinase family. The MAP kinase family has been classified into three subfamilies: extracellular signal-regulated protein kinases (ERKs), including ERK1 and ERK2 also known as p44MAPK and p42MAPK, respectively; stress-activated protein kinases, also termed c-Jun N-terminal protein kinases (JNKs), including JNK1 of 46 kDa and JNK2 of 55 kDa; and p\(38\) kinase, a homolog of the yeast high osmolarity glycerol response-1 kinase (1). ERKs phosphorylate and activate the transcription factor p62TCF/Elk-1, which forms a part of the ternary complex that regulates the transcriptional activity of the c-Fos promoter serum response element or SRE (2, 3). In contrast, JNKs phosphorylate two sites of the N-terminal transactivating domain of c-Jun (Ser-63 and Ser-73), ATF-2, and Elk-1, thereby increasing their transcriptional activity (4).

Recent data suggest that JNK is activated in response to cellular stress induced by certain DNA-damaging agents, including UV-C (5–7), ionizing radiation (8), cisplatin (9, 10), mitomycin C (9), adriamycin (11), etoposide (VP-16) (11), and alkylating agents such as vinblastine (11), N-methyl-N-nitro-N-nitrosoguanidine (5), 1-β-D-arabinofuranosylcytosine (12), and hydrogen peroxide (13). These observations suggest that the JNK cascade may mediate a physiological response to DNA damage such as induction of one or more DNA repair enzymes (10). However, the effect of certain DNA-damaging agents on ERK cascade remains unclear. In this study, we sought to determine whether JNK and/or ERK play a role in the cellular stress response to the chemotherapeutic agent cisplatin, which damages DNA through the formation of bifunctional platinum adducts using both Caov-3 human ovarian cancer cells, which are resistant to cisplatin, and A2780 human ovarian cancer cells, which are sensitive to cisplatin. Here, we provide evidence that cisplatin, but not transplatin, which does not readily damage DNA (14, 15), activates both JNK and ERK with different kinetics. Moreover, inhibition of both the JNK cascade and ERK cascade markedly decreased the cell viability following treatment with cisplatin but not with transplatin. Thus, both JNK and ERK are activated by cisplatin-induced DNA damage and are required for cell survival following cisplatin treatment.

EXPERIMENTAL PROCEDURES

Materials—Phorbol-12-myristate, 13-acetate (PMA) was purchased from Sigma. Sturosporine was purchased from Calbiochem. Hygromycin was purchased from Wako Pure Chemical Industries (Tokyo, Japan). ECL Western blotting detection reagents were obtained from Amersham Pharmacia Biotech. \(\gamma\)\(^{-32}\)P\(^\text{P}_{\text{ATP}}\) (3000 Ci/mmol) was obtained from NEN Life Science Products. Anti-phosphotyrosine (PY20) and mouse monoclonal anti-ERK antibodies were obtained from Up-

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¶ The abbreviations and trivial names used are: MAP, mitogen-activated protein; cisplatin, cis-diaminodichloroplatinum; transplatin, trans-diaminodichloroplatinum; ERK, extracellular signal-regulated (protein) kinase; JNK, c-Jun N-terminal protein kinase; dnJun, dominant negative c-Jun; PAGF, polyacrylamide gel electrophoresis; PKC, protein kinase C; PMA, phorbol-12-myristate, 13-acetate; MEK, mito-
state Biotechnology, Inc. (Lake Placid, NY). Rabbit polyclonal anti-
ERK1 antibody was obtained from Santa Cruz Biotechnology, Inc. (San-
ta Cruz, CA). PD98059 and the stress-activated protein kinase/JNK
assay kit, including N-terminal c-Jun fusion protein bound to glutath-
one-Sepharose beads and a phosphospecific c-Jun antibody, were ob-
tained from New England Biolabs (Beverly, MA). The Cell Titer-96 cell
cell proliferation assay was obtained from Promega (Madison, WI).

Cell Cultures—Human ovarian papillary adenocarcinoma cell line
(Caov-3) was obtained from American Type Culture Collection (Manas-
sas, VA). Human ovarian cancer A2780 cell line derived from a patient
prior to treatment was kindly provided by Dr. T. Tsuruo (Institute of
Molecular and Cellular Biosciences, Tokyo, Japan) and Drs. R. F. O’Reil-
ye and T. C. Hamilton (NCI, National Institutes of Health, Bethesda, MD)
(16, 17). The cells were cultured at 37 °C in Dulbecco’s modified Eagle’s
medium with 10% fetal bovine serum in a water-saturated atmosphere
of 95% O2 and 5% CO2.

Clone Selection—The dominant negative c-Jun (dnJun) expression
plasmid pLHcc-JUN (S63A,S73A) was constructed as described previ-
ously (18). Caov-3 and A2780 cells were transfected for 12 h in six-well
plate followed by a change of medium to fresh medium. The number of
plated or transplanted for 1 h after seeding test cells into 96-well
plates followed by a change of medium to fresh medium. The number of
surviving cells was determined 5 days later by determination of A590
nm of the dissolved formazan product after the addition of MTS for 1 h
as described by the manufacturer (Promega). All experiments were carried
out in quadruplicate, and the viability is expressed as the ratio of the
number of viable cells with cisplatin or transplatin treatment to that
without treatment.

Assay of ERK Activity—Cells were incubated in the absence of serum
for 16 h and then treated with various agents. They were then washed
twice with phosphate-buffered saline and lysed in ice-cold HNTG buffer
(50 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100,
1.5 mM MgCl2, 1 mM EDTA, 10 mM sodium pyrophosphate, 100 μM
sodium orthovanadate, 100 mM NaF, 10 μg/ml aprotinin, 10 μg/ml
leupeptin, and 1 mM phenylmethylsulfonyl fluoride) (21). The extracts
were centrifuged to remove cellular debris, and the protein content of
the supernatants was determined using the Bio-Rad protein assay
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the supernatants was determined using the Bio-Rad protein assay.

Assay of JNK Activity—Cells were incubated in the absence of serum
for 16 h and then treated with various agents. They were then washed
twice with phosphate-buffered saline and lysed in ice-cold HNTG buffer
(50 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100,
1.5 mM MgCl2, 1 mM EDTA, 10 mM sodium pyrophosphate, 100 μM
sodium orthovanadate, 100 mM NaF, 10 μg/ml aprotinin, 10 μg/ml
leupeptin, and 1 mM phenylmethylsulfonyl fluoride) (21). The extracts
were centrifuged to remove cellular debris, and the protein content of
the supernatants was determined using the Bio-Rad protein assay
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Cisplatin is a stereospecific activator of JNK. The dominant negative c-Jun
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as described by the manufacturer (Promega). All experiments were carried
out in quadruplicate, and the viability is expressed as the ratio of the
number of viable cells with cisplatin or transplatin treatment to that
without treatment.

Activation of JNK and ERK—To evaluate whether JNK is
activated by cisplatin in Caov-3 or A2780 human ovarian cancer
cell, cultured cells were exposed to cisplatin for the indi-
cated times (Fig. 1A) and at the indicated concentrations for 3 h
(Fig. 1B). Cell lysates were incubated with glutathione S-
transferase-c-Jun fusion protein, followed by precipitation and West-
er analysis using anti-phospho-c-Jun antibody. The activation
of JNK by cisplatin in Caov-3 cells was detectable at 1 h,
reached a broad plateau from 3 through 24 h, and declined
thereafter (Fig. 1A, upper panel). The activation of JNK by
cisplatin in A2780 cells was also detected at 1 h, reached
a plateau at 3 h, and declined thereafter (Fig. 1A, lower panel).
Cisplatin induced the activation of JNK in a dose-dependent
manner in Caov-3 (Fig. 1B, upper panel) and A2780 cells (Fig.
1B, lower panel). It is known that cisplatin but not transplatin
forms covalent cross-links between the N-7 position of adjacent
guanine or adenine-guanine residues (14, 15). The treatment
by transplatin at the same concentrations had no apparent
effect on JNK activation, whereas cisplatin induced JNK activa-
tion in Caov-3 (Fig. 1C) and A2780 (data not shown) cells.
These results indicate that only the DNA-damaging cisplatin
isomer activates JNK activity in both types of cells.

We next examined the effect of cisplatin on the activation of
ERK, which is a member of the MAP kinase family. Cultured
cells were exposed to cisplatin for the indicated times (Fig. 2A)
and at the indicated concentrations for 30 min (Fig. 2B).
Cell lysates were immunoprecipitated with anti-ERK antibody and
examined for ERK activity by assaying the incorporation of [32P]
imidazole. The cisplatin-dependent increase in
ERK activity displayed a biphasic time course; the activity
reached a maximum at 30 min, rapidly declined, increased
again after 3 h of cisplatin stimulation, and declined thereafter
in Caov-3 (Fig. 2A, upper panel) and A2780 (Fig. 2A, lower
panel) cells. Cisplatin-induced activation of ERK was maximal
at 100 μM and declined at higher concentrations in Caov-3 (Fig.
2B) and A2780 (data not shown) cells. Treatment by transpl-
Effects of Cisplatin on ERK and JNK

**Fig. 2. Cisplatin is a stereospecific activator of ERK.** Cells were grown in 100-mm dishes. A, Caov-3 and A2780 cells were treated with 100 μM cisplatin for the indicated times (lanes 2–6). B, Caov-3 cells were treated with the indicated concentrations of cisplatin for 30 min (lanes 2–7). C, Caov-3 cells were treated with 100 μM transplatin for the indicated times (lanes 2–4) or with 100 μM cisplatin for 30 min (lane 5). Lysates were subsequently immunoprecipitated with anti-ERK1 antisera, and the immunoprecipitates were incubated with [γ-32P]ATP in the presence of myelin basic protein (MBP) as described under “Experimental Procedures.” After the reactions were stopped by the addition of Laemmli sample buffer, samples were subjected to SDS-PAGE and autoradiographed.

**Fig. 3. The role of Ca2+ and PKC in the activation of ERK by cisplatin.** A, Caov-3 cells were grown in 100-mm dishes. PKC was down-regulated by incubation with 1 μM PMA for 24 h (lanes 3 and 6) or inhibited by a 10-min incubation with 1 μM staurosporine (lane 4). Extracellular and intracellular Ca2+ was chelated by a 15-min incubation with 3 mM EGTA (lane 8). The cells were treated with 100 μM cisplatin for 30 min (lanes 2–4 and 8) or 1 μM PMA for 10 min (lanes 5–7). B, A2780 cells were grown in 100-mm dishes. Extracellular and intracellular Ca2+ was chelated by a 15-min incubation with 3 mM EGTA (left panel, lane 3). PKC was inhibited by a 10-min incubation with 1 μM staurosporine (right panel, lanes 3 and 5). The cells were treated with 100 μM cisplatin for 30 min (left panel, lanes 2 and 3; right panel, lanes 2 and 3) or 1 μM PMA for 10 min (right panel, lanes 4 and 5). Activity of ERK was measured as described in the legend of Fig. 2. The experiments were repeated three times with essentially identical results. I.P., immunoprecipitation; I.B., immunoblot.

Cisplatin had no effect on ERK activation, whereas in parallel experiments cisplatin induced strong ERK activation in Caov-3 (Fig. 2C) and A2780 (data not shown) cells. Mitogenic stimuli activate ERK by increasing tyrosine and serine/threonine phosphorylation of the protein due to the activity of dual specificity MEK (27). Therefore, the cisplatin-dependent tyrosine phosphorylation of the predominant form of ERK was evaluated by antiphosphotyrosine Western analysis using the anti-ERK immunoprecipitates. The Caov-3 cells were treated with cisplatin, transplatin, or EGFP followed by lysis and evaluation of tyrosine phosphorylation of ERK (Fig. 2D). Both cisplatin and EGFP produced an increase in tyrosine phosphorylation of ERK, whereas transplatin had no effect. These results indicate that only the DNA-damaging cisplatin isomer again activates ERK activity and tyrosine phosphorylation.

**Involvement of Extracellular and Intracellular Ca2+ and Protein Kinase C in Cisplatin-induced Activation of ERK but Not of JNK—**We examined an upstream mediator in the cascade of the cisplatin-induced activation of ERK. Treatment with 3 mM EGTA for 15 min to eliminate extracellular Ca2+ and intracellular Ca2+ had no effect on the cisplatin-induced activation of JNK in Caov-3 and A2780 cells (Fig. 4, lanes 2, 4, 6, and 8). Moreover, treatment with 1 μM staurosporine for 10 min to inhibit protein kinase C had no effect on cisplatin-induced activation of JNK in Caov-3 and A2780 cells (Fig. 4, lanes 2, 3, 6, and 7). Thus, cisplatin-induced JNK activation is neither extracellular and intracellular Ca2+-dependent nor protein kinase C-dependent. These results suggest that the mechanism of cisplatin-induced activation of JNK is different from that of cisplatin-induced activation of ERK.

**Differential Activation of ERK and JNK Cascades by Cisplatin—**To confirm that cisplatin differentially activates JNK and ERK, the effect of a MEK inhibitor, PD98059, on the activation was tested in Caov-3 (Fig. 5) and A2780 (data not shown). This compound is relatively specific for MEK with no inhibitory activity against a number of other serine/threonine and tyrosine kinases (29–31). Although the MEK inhibitor (100 μM) largely repressed the ERK activation induced by cisplatin for 30 min (Fig. 5A) or 3 h (data not shown), this compound had no apparent effect on cisplatin-induced JNK activation (Fig. 5B), suggesting that the activation of JNK by cisplatin is independent of the activation of ERK. To rule out the possibility that the second phase of ERK activation is caused by JNK activation, we examined whether ERK is activated by cisplatin in clonal lines of Caov-3 cells, which stably expressed a dominant negative inhibitor (32, 33) of the JNK cascade, dnJun. (Fig. 5C). The dnJun mutant cannot be phosphorylated at the N-terminal
serine residues due to substitution of serines 63 and 73 by alanine, thereby blocking the enhanced transactivation promoted by JNK-dependent phosphorylation of these sites (32, 33). Thus, dnJun blocks c-Jun phosphorylation-dependent events of the JNK cascade (18, 32, 33). Expression of dnJun has no effect on either basal AP-1 activity (32, 33) or on the enzyme activity of JNK(data not shown) but does inhibit phosphorylation-dependent activation of transcription (32–34). The ERK activity induced by cisplatin for 3 h in dnJun-expressing cells appeared to be similar to that in an empty vector-expressing cells (Fig. 5C), supporting the differential activation of ERK and JNK cascades.

Dominant Negative c-Jun Sensitizes Caov-3 Cells to Cisplatin but Not to Transplatin—The effect of cisplatin treatment on the viability of a representative dnJun-expressing clonal line was compared with that of an empty vector-expressing control line (Fig. 6A). The viability of the control Caov-3 cells remained unaffected by increasing concentrations of cisplatin to >100 µM. Extended titerations revealed IC₅₀ values of 380 and 412 µM for the parental cells and empty vector-expressing control lines, respectively (Table I). In contrast, the dnJun-expressing cells exhibited an IC₅₀ as low as 50 µM or over 7.6-fold more sensitive to cisplatin than the control cells (Fig. 6A, Table I). Transplatin had no discernible effect on the dnJun-expressing line at concentrations where the viability following treatment with cisplatin was less than 20% (Fig. 6B). In extended titrations, no significant effect was observed with transplatin even at 250 µM, indicating that the requirement for sensitization by dnJun depends upon the stereospecific DNA-binding properties of cisplatin, consistent with the results in the activation of JNK.

Expression of wild-type c-Jun did not affect the sensitivity to cisplatin, compared with the control line (Fig. 6A). Thus, the sensitization to cisplatin observed in the dnJun-expressing cells appeared to be due to the interference with the activation of c-Jun by JNK.

PD98059 Sensitizes Caov-3 Cells to Cisplatin but Not to Transplatin—We next examined whether the ERK cascade is also required for the cell viability following cisplatin treatment of Caov-3 cells. The cells pretreated with PD98059 exhibited an IC₅₀ as low as 39 µM, or over 9.7-fold more sensitive to cisplatin than the untreated cells (Fig. 7A). Transplatin had no discernible effect (Fig. 7B), indicating that the requirements for sensitization by PD98059 also depend upon the stereospecific DNA-binding properties of cisplatin, similar to the results in the activation of ERK. Thus, the sensitization to cisplatin observed in cells treated with PD98059 appeared to be due to the interference with activated ERK.

Dominant Negative c-Jun or PD98059 Sensitizes A2780 Cells—Next, we examined the effect of interference with either the JNK or ERK cascade on cell viability by cisplatin by using A2780 cells. We developed clonal lines of A2780 cells, which stably expressed dnJun. The IC₅₀ value of the parental cells was 84 ± 4 µM (Table I). In contrast, the dnJun-expressing cells and the cells pretreated with PD98059 exhibited an IC₅₀ as low as 20 and 48 µM, or over 4.2- and 1.8-fold more sensitive to cisplatin than the control cells, respectively (Fig. 8, Table I). Thus, the expression of dnJun or the treatment of PD98059 also sensitized A2780 cells to cisplatin.

Effect of PD98059 Pretreatment in Cells with or without Inhibition of JNK Cascade—To examine whether JNK and ERK cascades are differentially involved in the cell survival...
following cisplatin treatment, the effect of PD98059 on the cell viability in the empty vector-expressing control cells was compared with that in the dnJun-expressing cell line following cisplatin treatment. No additive effect was detected when the both cascades were inhibited in Caov-3 (Fig. 9) and A2780 (data not shown) cells. The results suggested that activation of both JNK and ERK cascades are needed to retain cell viability under cisplatin treatment.

**DISCUSSION**

These studies show that both JNK and ERK cascades are activated by cisplatin-induced DNA damage and are required for the cell viability following cisplatin treatment in both cisplatin-resistant and -sensitive cells. We have used a nonphosphorylatable dominant negative c-Jun, dnJun, where the two serine residues at positions 63 and 73 are replaced by two alanine residues, to dissect the JNK cascade and a specific inhibitor of MEK (PD98059) to block the activation of ERK cascade. dnJun has been characterized and successfully used in independent studies using highly characterized antisense reagents. However, the specific role of JNK and ERK in cisplatin resistance is still unclear.

Fig. 7. Treatment with PD98059 sensitizes Caov-3 cells to cisplatin. Cell viability assays were conducted using parental cells (○), dnJun-expressing cells (●), and cells pretreated with 100 nM PD98059 for 30 min (▲) following the indicated concentrations of cisplatin treatments as described under “Experimental Procedures.”

Fig. 8. Dominant negative c-Jun or treatment with PD98059 sensitzes A2780 cells to cisplatin. Cell viability assays were conducted using parental cells (○), dnJun-expressing cells (●), and cells pretreated with 100 nM PD98059 for 30 min (▲) following the indicated concentrations of cisplatin treatments as described under “Experimental Procedures.”

Fig. 9. Effect of PD98059 pretreatment in cells with or without inhibition of JNK cascade. Caov-3 cells expressing empty vector (circos) or dnJun (●) were pretreated with 100 nM PD98059, followed by cisplatin treatment, and cell viability assays were carried out as described under “Experimental Procedures.”

The stimulation of cell proliferation by growth factors involves a coordinated series of signaling events that serve to transduce extracellular signals across the plasma membrane and into the nucleus, thereby inducing the expression of a variety of genes that are important for regulating cell cycle. Two such genes are the protooncogenes c-fos and c-jun, which are prototypes for a family of transcription factors that dimerize to form the transcription factor complex called AP-1, which transactivates many kinds of genes that have a TRE site in their promoter (34). The binding of c-Fos and c-Jun to TRE is controlled by the activation of specific kinase cascades that is regulated by growth factors. One important downstream biochemical event that occurs after ligation of many growth-promoting receptors is the activation of members of the MAP kinase family, including ERK and JNK (1). ERKs have been reported to phosphorylate the ternary complex factor, Elk-1, which controls the expression of the c-fos gene (36, 37). It has been demonstrated that JNK phosphorylates c-Jun and ATF-2 at the putative regulatory amino-terminal serine residues and increases their transcriptional activities (4, 5, 24) including increased transcription and expression from the c-Jun and ATF-2 genes themselves (66, 67). Moreover, JNK has been reported to activate Elk-1, resulting in the increase in c-fos gene expression. Therefore, ERK and JNK cascades are agonist-stimulated protein kinase cascades that transduce signals into the nucleus to modulate the expression of c-Fos (ERK), and c-Jun (JNK). The ERK cascade is strongly activated by growth and differentiation factors, and sustained activation is thought to be an important signal for promoting cell proliferation and differentiation (38–42). The JNK cascade is also activated by cellular stresses (24, 43). These observations suggest the existence of parallel cascades leading to activation of either ERK or JNK.

Although it has been previously shown that JNK activation occurs following the cisplatin-induced DNA damage (9, 10), until recently there had not been studies addressing the effect of cisplatin on ERK activation. It was reported that many
effects of cisplatin remains unknown. JNK phosphorylates c-Jun function with dominant negative SEK protects against cisplatin (58, 59), our data demonstrating that treatment of cells upstream of JNK activation by cisplatin. Therefore, there is a possibility that these protooncogenes exist independently activated in cisplatin-treated Caov-3 and A2780 cells.

What is the upstream mediator of JNK and ERK activation by cisplatin? In most cases (45, 46), PKC and Ca²⁺ are well known to stimulate ERK activity. However, in endothelin-1-stimulated Rat-1 cells, JNK, but not ERK, activation is inhibited by chelation of Ca²⁺ and by down-regulation of PKC (47). Similarly, in cardiac myocytes, activation of JNK by angiotensin II was strongly suppressed by down-regulation of PKC or by chelation of intracellular Ca²⁺ (48). On the other hand, in GN4 rat liver epithelial cells, angiotensin II activates JNK in a Ca²⁺-dependent, PKC-independent manner (49). In this study, cisplatin-induced ERK activation was mediated by extracellular and intracellular Ca²⁺ and by protein kinase C, but cisplatin-induced JNK activation was not (Fig. 3 and 4). Thus, the upstream mediator involved in the JNK activation by cisplatin may be different from that involved in the ERK activation. Recently, it has been shown that RAS mediates cell proliferation and cell transformation not only through RAF/ERK but through other cascades involving protooncogenes of the Rho family, Rac1 and CDC42 (50, 52–54). These latter two protooncogenes have been reported to stimulate the activity of JNK cascade (43, 54) and to mediate RAS transformation (56). Therefore, there is a possibility that these protooncogenes exist upstream of JNK activation by cisplatin.

Since it is reported that the ERK cascade plays a role in opposing cell death stimuli (57) and that interruption of the ERK cascade sensitizes cells to apoptosis induced by certain agents (58, 59), our data demonstrating that treatment of cells by PD98059 promoted sensitivity to cisplatin confirms a protective role of the ERK cascade from cell death stimuli. On the other hand, it is well known that the JNK cascade is activated by cellular stresses (5–13, 24, 43, 60, 61) and interruption of c-Jun function with dominant negative SEK protects against cisplatin (62), indicating a functional role of the JNK cascade in mediating drug-induced cell death. However, our results demonstrated that the JNK cascade was also required to maintain the cell viability following cisplatin treatment, consistent with the results in a previous report (10). Although little is known regarding a role of JNK activation in cell proliferation and transformation of human tumor cells, an essential role of JNK cascade in growth stimulation by EGF in human A549 lung carcinoma cells is reported (35). However, the role of JNK in EGF-stimulated growth or the viability following the cytotoxic effects of cisplatin remains unknown. JNK phosphorylates c-Jun at its N-terminal activation domain at serine residues 63 and 73, leading to enhanced transcriptional activity required for the transformation of primary rat embryo fibroblasts in cooperation with activated RAS (32). By using dnJun, it may be possible to inhibit the transformation of rat embryo fibroblast cells by activated RAS. In addition to JNK activation of AP-1, a transcription factor consisting of c-Fos/c-Jun heterodimers or c-Jun/c-Jun homodimers, JNK also phosphorylates ATF-2 (4, 63, 64) and Elk-1 (65). AP-1 and ATF-2 are important transcription factors regulating numerous genes implicated in cell growth, transformation, differentiation, and DNA repair (30, 66–68). Several enzymes known to be involved in repair of DNA-cisplatin adducts and implicated in cisplatin resistance (13) contain ATF/cAMP-response element-binding protein sites in their promoters including DNA polymerase β (69, 70), topoisomerase I (71, 72), and proliferating cell nuclear antigen, an accessory protein of DNA polymerase δ (55, 73). Moreover, transcription of these genes is known to be activated through the ATF/cAMP-response element-binding protein sites upon stimulation by genotoxic agents (55, 69–73). On the other hand, the ERK cascade is strongly activated by growth factors, and sustained activation of ERK is thought to be an important signal for promoting cell proliferation by transactivation of AP-1 function. Although it has never been reported that DNA repair enzymes contain AP-1 sites in their promoter, the existence of AP-1 sites in the promoter of the multidrug resistance gene has been reported (51).

What is the difference in the signaling cascade induced by cisplatin between sensitive and resistant cells? In the cells sensitive to cisplatin, cisplatin induces a persistent activation of JNK, not of ERK, suggesting that a prolonged activation of JNK probably results from unrepaired DNA damage and that the absence of ERK cascade activation promotes cell death (44). However, this study identified that cisplatin differentially activated the JNK and ERK cascade, and both cascades seem to be necessary to maintain the cell viability following cisplatin treatment in both sensitive and resistant cells. Thus, activation of JNK and ERK cascades by cisplatin may have a physiological role in regulating cell viability following genotoxic stress by treatment with cisplatin. To examine whether each cascade is independently involved in cell viability, we compared the effect of PD98059 on the cell viability following cisplatin treatment between dnJun- and empty vector-expressing lines. We did not detect any difference in the effect of PD98059 with or without dnJun expression, suggesting that both cascades are independently involved and may share a crucial downstream step such as the formation of an active c-Jun/c-Fos complex. These results suggest that transactivation of AP-1 sites that are activated by both ERK and JNK cascades might be necessary for repair of cisplatin treatment.

It remains to be determined whether other MAP kinase family members such as p38 or the newly described stress-activated protein kinase 3 (1) are also activated by cisplatin and whether other JNK substrates such as ATF-2 are affected. This study provides the first evidence suggesting a potential physiological role of the differential activation of JNK and ERK cascade for the cell viability following cisplatin-induced DNA damage.

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