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Cisplatin-induced Activation of Mitogen-activated Protein Kinases in Ovarian Carcinoma Cells: Inhibition of Extracellular Signal-regulated Kinase Activity Increases Sensitivity to Cisplatin

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Abstract

Cisplatin treatment activates multiple signal transduction pathways, which can lead to several cellular responses including cell cycle arrest, DNA repair, survival, or apoptosis. We investigated the response of the mitogen-activated protein kinases, extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-Jun-N-terminal kinase 1 (JNK1), and p38, to cisplatin treatment in the ovarian carcinoma cell line SK-OV-3. Cisplatin caused a late and prolonged induction in a dose-dependent manner of both ERK1/2 and JNK1 activity. ERK1/2 and JNK1 activities continued to increase in magnitude up to 24 h following initiation of cisplatin treatment. In contrast, cisplatin treatment had no effect on p38 activity. Transplatin failed to induce either ERK1/2 or JNK1 at 24 h, which suggests that the activation of these kinases was dependent on cisplatin-specific DNA damage. Treatment with cycloheximide resulted in inhibition of cisplatin-induced ERK1/2 activation, demonstrating that ERK1/2 activity induced by cisplatin was dependent on de novo protein synthesis. Furthermore, inhibition of cisplatin-induced ERK1/2 activity by PD 98059 caused enhanced cisplatin cytotoxicity. Similar enhanced cytotoxic effects of cisplatin were also observed following treatment with PD 98059 in the ovarian carcinoma cell line UCI 101. These observations indicate that ERK1/2 activation induced by cisplatin partially protects cells from cisplatin cytotoxicity. Continued investigation into the mechanism by which the ERK pathway and other signal transduction pathways modulate the response to cisplatin may be helpful in the development of new strategies for improving the therapeutic use of platinum drugs.

Introduction

Platinum-containing drugs, including cisplatin and carboplatin, are widely used in the treatment of solid tumors such as ovarian, testicular, head and neck, bladder, and lung cancer. One of the major limitations to the use of these drugs is the acquisition of resistance to initially responsive tumors (1). Possible mechanisms of acquired resistance include altered cellular drug transport, enhanced intracellular detoxification, increased DNA repair, and enhanced tolerance to platinum-DNA damage (2–6). Understanding the cellular responses to platinum-based drugs is critical for determining mechanisms of drug resistance and for allowing the development of therapeutic approaches for increasing the effectiveness of cisplatin or carboplatin treatment.

Cytotoxicity produced by cisplatin and its analogues has been shown to be a consequence of the DNA damage caused by formation of cisplatin-DNA adducts (1). Similar to other DNA damaging agents, cisplatin produces several cellular responses, including the induction of DNA damage-inducible genes such as gadd153, gadd45, p21, and c-jun, and the activation of the p53 pathway (8).

Genotoxic stress induces multiple signal transduction pathways, among which are members of the MAP kinase pathways (4). The MAP kinase pathways are parallel cascades of structurally related serine/threonine kinases that serve to integrate numerous extracellular signals, resulting in regulation of cell proliferation, differentiation, and cell survival (for reviews, see Refs. 9–13). ERK, JNK, and p38 are the terminal enzymes in three major kinase cascades within the MAP kinase family (10, 11, 14). Activation of ERK1 and ERK2 (ERK1/2), by a variety of mitogenic receptors (e.g., receptors for epidermal growth factor, insulin-like growth factor, and platelet-derived growth factor) leads to the production of proteins required for cell growth or differentiation (10, 13). ERK activation usually involves participation of Ras and Raf oncoproteins and activation of MEK, a dual-specificity kinase that phosphorylates ERK1 and ERK2 (10, 12–14). Activated ERK phosphorylates several substrates, including Elk-1 and ATF2 (10, 11). In contrast, the cascades involving JNK and p38 are activated primarily by environmental stress such as UV light, heat shock, and hypoxia or by inflammatory cytokines such as tumor necrosis factor α and FAS ligand (10, 11, 13, 14). JNK (JNK1 and JNK2) is regulated by a kinase cascade similar to the ERK pathway. JNK-activating kinase, which phosphorylates JNK, is in turn

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3 The abbreviations used are: MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; JNK, c-Jun-N-terminal kinase; MEK, MAP/ERK kinase; MEKK, MEK kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; PMS, phenazine methosulfate; TLB, Triton lysis buffer.

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activated by MEKK. JNK phosphorylates a number of transcriptional factors, among them c-Jun, Elk-1, and ATF2 (10–12).

In the present study, we investigated the effects of cisplatin treatment on ERK1/2, JNK1, and p38 kinase activity in the ovarian carcinoma cell line SK-OV-3. We demonstrated that late activation of ERK1/2 and JNK1 occurred in a dose- and time-dependent manner following cisplatin treatment. Moreover, inhibition of cisplatin-induced ERK1/2 activity was associated with increased sensitivity to cisplatin. These observations indicate that both ERK1/2 and JNK1 are activated by cisplatin-induced DNA damage and that the induction of ERK1/2 serves a protective role against the cytotoxic effects of cisplatin.

Materials and Methods

Cell Lines. The human epithelial adenocarcinoma ovarian cell line SK-OV-3 (a gift from Dr. P. Terranova, University of Kansas Medical Center, Kansas City, Kansas) and UCI 101 (a gift from Dr. P. Carpenter, University of California at Irvine, Orange, CA; Ref. 15) were maintained in RPMI 1640 containing penicillin/streptomycin/glutamine and 10% fetal bovine serum (Life Technologies, Gaithersburg, MD) in a humidified atmosphere of 5% CO2 at 37°C.

Cytotoxicity Assay. Cells at 70–80% confluency were trypsinized (0.25% trypsin with 1 mM EDTA), washed, resuspended in growth medium, and plated in 96-well plates with 0.2 ml of the 104 cell/ml cell suspension seeded in each well. After overnight incubation, cells were treated with specific reagents for 24 h or 3 days. The cell number was determined using a modified MTT assay, the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega Corp., Madison, WI) containing MTS and PMS (16). The MTS/PMS solution (20 μl) was added to each well, the cells were incubated for 3 h, and the absorbance was measured at 490 nm on an MRX microplate reader (Dynex Technologies, Inc., Chantilly, VA). The amount of survival was calculated as the absorbance ratio of treated to untreated cells. Each experimental treatment was performed in quadruplicate in three independent experiments.

Preparation of Cellular Extracts. Cells were grown in 100-mm Petri dishes for 72 h to 70–80% confluency, followed by a 24-h treatment with either 0.01–100 μg/ml cis-platinum (II)diamine dichloride (cisplatin; Sigma, St. Louis, MO) dissolved in PBS or 10 μg/ml trans-platinum(II)diamine dichloride (transplatin; Sigma) dissolved in PBS. In studies using cycloheximide or PD 98059, cells were pretreated for 1 h with either 1 μg/ml cycloheximide (Sigma) dissolved in PBS or 0.1 mM PD 98059 (New England Biolabs, Beverly, MA) dissolved in DMSO (DMSO concentrations <0.2%). Cells were washed with PBS and lysed in 500 μl of ice-cold TLB [20 mM Tris (pH 7.4), with 137 mM NaCl, 25 mM β-glycerophosphate, 2 mM EDTA, 1 mM sodium vanadate, 2 mM NaP, 1% Triton X-100, and 10% glycerol]. Portions of the same cellular extracts were used for JNK1, ERK1/2, and p38 immune-complex kinase assays and for Western blot analysis.

ERK1/2 Immune-Complex Kinase Assay. Clarified cellular extracts were immunoprecipitated with anti-ERK1 and anti-ERK2 antibodies (C-16 and C-14; Santa Cruz Biotechnology, Santa Cruz, CA) prebound to Protein A/G Plus-Agarose in TLB+ (TLB with the following protease inhibitors: 1 mM PMSF, 5 μg/ml leupeptin, 5 μg/ml aprotinin, 2 mM benzamidine, and 0.5 mM DTT) for 2 h at 4°C. Immune-complex kinase assays (17) were performed in a final volume of 28 μl of ERK assay buffer [40 mM Tris (pH 7.4), 20 mM MgCl2, 2 mM MnCl2, 0.5 mM DTT] with 11 μg of myelin basic protein (Sigma) as a substrate, 30 μM unlabeled ATP, and 9 μCi of [γ-32P]ATP (Amersham Corp., Arlington Heights, IL). Reactions were conducted for 30 min at 37°C and terminated by addition of 2X SDS-PAGE sample buffer. Samples were resolved on 14% SDS-PAGE (Novex, San Diego, CA), stained, dried, and autoradiographed. The kinase activity was quantified using PhosphorImager analysis.

JNK1 Immune-Complex Kinase Assay. Clarified cellular extracts were immunoprecipitated with anti-JNK1 antibody (C-17; Santa Cruz Biotechnology) prebound to Protein A/G Plus-Agarose (Santa Cruz Biotechnology) in TLB+ for 2 h at 4°C. Immune-complex kinase assays (18) were performed in a final volume of 30 μl of JNK assay buffer [25 mM HEPES (pH 7.4), 25 mM β-glycerophosphate, 25 mM MgCl2, 0.1 mM sodium vanadate, 0.5 mM DTT] with 8 μg of GST-c-Jun (1–79; a gift from Dr. R. Davis, Howard Hughes Medical Institute, University of Massachusetts Medical Center, Worcester, MA) as a substrate, 30 μM unlabeled ATP, and 9 μCi of [γ-32P]ATP. Reactions were conducted for 15 min at room temperature and terminated by addition of 2X SDS-PAGE sample buffer. Samples were resolved on 12% SDS-PAGE, stained, dried, and autoradiographed. The kinase activity was quantified as described above.

p38 Immune-Complex Kinase Assay. Clarified cellular extracts were immunoprecipitated with anti-p38 antibody (C-20; Santa Cruz Biotechnology) prebound to Protein A/G Plus-Agarose in TLB+ for 2 h at 4°C. Immune-complex kinase assays (19) were performed in a final volume of 27 μl of p38 assay buffer [25 mM HEPES (pH 7.4), 25 mM β-glycerophosphate, 25 mM MgCl2, 0.1 mM sodium vanadate, 0.5 mM DTT] with 10 μg of myelin basic protein as a substrate, 30 μM unlabeled ATP, and 9 μCi of [γ-32P]ATP. Reactions were conducted for 30 min at 30°C and terminated by addition of 2X SDS-PAGE sample buffer. Samples were resolved on 14% SDS-PAGE, stained, dried, and autoradiographed. The kinase activity was quantified as described above.

Western Blot Analysis. Clarified cellular extracts (20 μg) were resolved on 10% SDS-PAGE under denatured reducing conditions and transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat milk, washed, and incubated with either anti-ERK1 and anti-ERK2 (C-16 and C-14) or anti-JNK1 (C-17) primary antibodies in 0.5% nonfat milk (1:1000). The membranes were then incubated with peroxidase conjugated secondary antibodies (1:10000; Sigma), washed, and visualized by enhanced chemiluminescence (Amersham).

Results

Dose Response and Time Course of Cisplatin Induction of MAP Kinases. The dose response of ERK1/2 and JNK1 to cisplatin is illustrated in Fig. 1. Increases above basal levels for ERK1/2 activity were first observed at 5 μg/ml cisplatin, whereas JNK1 activity began to rise at slightly lower concen-
ERK1/2 activity increased to 2.4-fold above basal activity, compared with a 10-fold increase in JNK1 activity with 25 μg/ml cisplatin. For the remaining studies, 10 μg/ml cisplatin was used to evaluate responses to cisplatin. This concentration was chosen because it is the IC90 (at 3-day exposure) of the cell line and also is a concentration that efficiently induces both JNK1 and ERK1/2 activity.

There was no induction of p38 over a 24-h period following treatment with 10 μg/ml cisplatin; therefore, no additional studies were pursued with p38 (Fig. 2). ERK1/2 and JNK1 activities were induced by cisplatin in a time-dependent manner (Fig. 2). Activation of JNK1 above the basal level occurred by 4 h and increased to 11-fold by 24 h. Cisplatin-induced ERK1/2 activation occurred at 4 h and increased to 6-fold induction by 24 h. The activities of both ERK1/2 and JNK1 decreased by 48 h (data not shown), suggesting that the peak induction time was 24 h. We did not observe any induction of both ERK1/2 and JNK1 activity after treatment with 10 μg/ml cisplatin for short periods of time up to 60 min (Fig. 2, inset). The increased activities of ERK1/2 and JNK1 at 24 h were not associated with any significant change in the levels of proteins as demonstrated by Western blot analysis (Fig. 3).

Requirement of Cisplatin-DNA Adducts for Activation of ERK1/2 and JNK1. The late induction of both ERK1/2 and JNK1 activities suggested that their activation may occur after the formation of cisplatin-DNA adducts. To verify this hypothesis, transplatin, an analogue of cisplatin, was examined for its ability to induce ERK1/2 and JNK1. Transplatin forms DNA adducts that are distinct from those formed by cisplatin and is less cytotoxic than cisplatin at a given concentration (20). Following exposure for 24 h, little effect on cell viability was observed with 10 μg/ml transplatin compared with an approximately 40–50% decrease in survival of cells in the presence of 10 μg/ml cisplatin (Fig. 4). Evidence of apoptosis, as indicated by the presence of a DNA ladder, was observed with the cisplatin treatment, but not with transplatin (data not shown). Transplatin failed to induce ERK1/2 or JNK1 activity (Fig. 5), suggesting that activation of both kinases was dependent on the
specific damage caused by cisplatin-DNA adducts or the downstream effects of cisplatin-induced DNA damage.

**Inhibition of Cisplatin-induced ERK1/2 Activity by Cycloheximide.** Because a significant delay in time was observed following initiation of cisplatin treatment before induction of ERK1/2 and JNK1 activities, it was possible that the increase in kinase activity was secondary to de novo protein synthesis. To determine whether protein synthesis was required for cisplatin-induced JNK1 and ERK1/2 activation, the cell line was pretreated with cycloheximide. Cycloheximide alone (1 µg/ml) decreased cell survival to 63%, as determined by the MTT assay, which was similar to the effects of 10 µg/ml of cisplatin (Fig. 4). Clonogenic assays also indicated that cycloheximide inhibited cell proliferation by ~50%; however, analysis of DNA fragmentation showed no evidence that cycloheximide caused apoptosis (data not shown). The combination of cycloheximide and cisplatin resulted in lower cell survival than with either treatment alone (38% survival, Fig. 4). Cycloheximide alone had no effect on ERK1/2 or JNK1 activity (Fig. 5). ERK1/2 induction by cisplatin was inhibited completely by cycloheximide. In contrast, the combination of cycloheximide and cisplatin treatment produced a slightly higher, although not statistically significant, increase in JNK1 activity compared with...
cisplatin alone (13-fold and 11-fold, respectively). Thus, the cisplatin-induced ERK1/2, but not JNK1, activation was dependent on de novo protein synthesis.

**Enhanced Sensitivity to Cisplatin following ERK1/2 Inhibition.** Because the enhanced sensitivity to cisplatin in the presence of cycloheximide was accompanied by inhibition of cisplatin-induced ERK1/2 activity, the question arose as to whether ERK1/2 activity could modulate cellular sensitivity to the drug. To examine this possibility, ERK1/2 activation by cisplatin was inhibited specifically by the MEK1 inhibitor PD 98059. Phosphorylation and activation of MEK1, the immediate upstream kinase of ERK1/2, is inhibited by PD 98059 (21, 22). The inhibition of MEK1 activation consequently results in the inhibition of activation of its substrate, ERK1/2. Because PD 98059 functions by inhibiting phosphorylation of MEK1, PD 98059 does not affect the basal activities of MEK1 or ERK1/2 (22).

The effects of PD 98059 on ERK1/2 and JNK1 activities induced by cisplatin are illustrated in Fig. 6. At the concentration of 100 μM, PD 98059 completely inhibited the cisplatin-induced ERK1/2 activity but had little effect on the JNK1/2 activity. Lower concentrations of PD 98059 (10 μM and 50 μM) did not significantly affect the cisplatin-induced ERK1/2 activation (data not shown). DMSO, the solvent for PD 98059, slightly inhibited cisplatin-induced JNK1 activity, from 8.8- to 7.2-fold, but had no effect on cisplatin-induced ERK1/2 activity.

Pretreatment with PD 98059 resulted in enhanced sensitivity to cisplatin cytotoxicity (Fig. 7). Statistically significant decreases in cell survival were observed with 100 μM PD 98059 at 5 and 10 μg/ml of cisplatin (ANOVA; *P* < 0.05), and approached significance (*P* = 0.07) at 1.0 μg/ml cisplatin. At concentrations of 5 and 10 μg/ml, the percentage of surviving cells was 50% lower in the presence of PD 98059 compared with cisplatin treatment alone (5 μg/ml 62 and 31%; 10 μg/ml 34 and 16%). Lower concentrations of PD 98059 (25 and 50 μM) also contributed to the enhancement of cisplatin cytotoxicity, but the maximum effect was observed with 100 μM PD 98059 (data not shown).

To verify that the enhanced sensitivity to cisplatin in the presence of PD 98059 was not restricted to the SK-OV-3 cell line, the ovarian carcinoma cell line UCl 101 was also treated with PD 98059. Similar results of enhanced cisplatin cytotoxicity were observed in UCI 101 cells in the presence of PD 98059 (Fig. 8). In this cell line, we also demonstrated that cisplatin induced both ERK1/2 and JNK1 activation in a time- and concentration-dependent manner, but not p38 (data not shown). Pretreatment with PD 98059 led to a decrease of the cisplatin-induced ERK1/2 activity, but had little effect on JNK1 activity (Fig. 9).

**Discussion**

Genotoxic stress induces numerous signaling pathways that can influence cell growth and cytotoxicity. This is the first study that has characterized extensively the responses of ERK1/2, JNK1 and p38 to cisplatin. We demonstrated that both the ERK1/2 and JNK1 pathways are among the signal transduction pathways that are induced by cisplatin in the ovarian carcinoma. In contrast to the usual rapid and transient activation of these pathways in response to various other stimuli such as growth factors, UV damage, or cytokines (23), both ERK1/2 and JNK1 induction was delayed for several hours following initiation of cisplatin treatment. In addition, the ERK1/2 and JNK1 activities remained elevated for a prolonged period of time (24 h after exposure). The increases in activity were not associated with changes in protein levels for either ERK1/2 or JNK1.
The time course for activation of ERK1/2 and JNK1 was consistent with post-DNA damage induction. Formation of cross-linking cisplatin-DNA adducts involves at least two stages (24). The initial formation of a mono-adduct, usually bound to the N-7 position of guanine, is followed by a slower step, in which an intrastrand or interstrand cross-link is formed. The second reaction may require hours to complete. In the present study, both ERK1/2 and JNK1 activities were not significantly induced by cisplatin until approximately 4 h after initiation of treatment, an observation that is consistent with activation occurring after DNA adduct formation.

Fig. 7 Effects of PD 98059 on cisplatin-induced cytotoxicity in SK-OV-3 cells. Cells were pretreated with PD 98059 (0.1 mM) or the solvent DMSO (0.1%) for 1 h prior to 3-day exposure to the indicated concentrations of cisplatin. Cytotoxicity assays were performed with a modified MTT assay: white columns, cisplatin only; striped columns, DMSO and cisplatin; black columns, PD 98059 and cisplatin. Results represent the mean of three experiments performed with quadruplicate cultures; bars, SE.

By demonstrating the lack of induction of both ERK1/2 and JNK1 activities by transplatin, we provided indirect evidence that activation of both kinases was dependent on cisplatin-specific DNA damage or the following downstream effects. Transplatin is an analogue of cisplatin that does not form the 1,2-d(GpG) or 1,2-d(ApG) adducts, which make up more than 90% of the adducts formed by cisplatin (20). Furthermore, in the experiments with cycloheximide, we showed that ERK1/2, but not JNK1, activation was dependent on de novo protein synthesis. This data suggest that activation of each kinase was likely initiated by different cellular stimuli or through separate cellular mechanisms.

Limited information was available previously on the effect of cisplatin on JNK activation. A weak induction of JNK activity in response to cisplatin was reported by Liu et al. (25). A higher level of JNK activation, with similar kinetics of induction to those that we observed, was described in Pam 212 mouse keratinocytes (26). In addition, activation of the JNK pathway by cisplatin in T98G glioblastoma cells was reported recently by Potapova et al. (27).

JNK activation by other stress stimuli, such as γ radiation, has been associated with apoptosis (28). Likewise, apoptosis caused by nerve growth factor withdrawal in PC12 pheochromocytoma cells can be partially blocked by overexpression of a dominant negative SEK1, a component of the JNK pathway (29).

The role of JNK activation by cisplatin in the cellular response to cisplatin remains unclear. Cisplatin-induced JNK1 activation appeared to be associated with cell death in Pam 212 mouse keratinocytes (26). In contrast, inhibition of the JNK pathway by expression of a dominant negative mutant c-Jun in T98G glioblastoma cells resulted in blocked DNA repair and decreased viability following cisplatin treatment. These later results suggested that cisplatin-induced JNK activation was required for DNA repair and cell survival following cisplatin.
treatment (27). Ongoing investigations in our laboratory are directed at verifying the role of JNK1 activation in either apoptosis or cell survival following cisplatin treatment in ovarian carcinoma.

Induction of ERK1/2 activity in response to cisplatin had been reported by one other group of investigators. Sánchez-Perez et al. (26) reported a weak and transient activation of ERK following cisplatin treatment in Pam 212 mouse keratinocytes. The weak response, which disappeared after 3 h, was considered insignificant by the investigators. In contrast, in our study, not only did cisplatin induce a significant increase in ERK1/2 activity, but the activation occurred late and remained elevated at 24 h after initiation of treatment.

Because the role of ERK activation in the cellular response to cisplatin had not been examined previously, we inhibited the cisplatin-induced ERK1/2 activation with the specific MEK1 inhibitor PD 98059. Treatment with PD 98059 led to enhanced cisplatin cytotoxicity in SK-OV-3 cell line. These results demonstrated that cisplatin-induced ERK1/2 activation provided the cells with partial protection against the cytotoxic effects of cisplatin. Enhancement of cisplatin cytotoxicity in the presence of PD 98059 was also observed in another ovarian carcinoma cell line, UCI 101. Therefore, the protective effect against the cytotoxic effects of cisplatin associated with induction of ERK1/2 activity was not restricted to the SK-OV-3 cell line.

Activation of ERK is critical not only in cell proliferation and differentiation (9–11), but can also be important as a survival signal (12, 29). Opposing effects of ERK and JNK/p38 pathways have been demonstrated on apoptosis caused by withdrawal of nerve growth factor from PC12 pheochromocytoma cells (29). Because a decrease in ERK activity and an increase in JNK activity were correlated with apoptosis in the PC12 cells, the findings suggested that ERK activation may provide a survival signal, whereas JNK activation provides an apoptotic signal. The authors of this study concluded that the balance between the two signaling pathways may be critical in determining cell survival or cell death. Our finding of increased sensitivity of ovarian carcinoma cells to the cytotoxic effects of cisplatin following inhibition of ERK induction demonstrated that ERK activation also provides a survival signal following cisplatin treatment. Similar finding showing enhanced sensitivity to apoptotic signals upon interruption of the ERK pathway have been described recently. Jarvis et al. (30) showed that ara-C stimulated ERK activation in human leukemia cells HL-60 and that PD 98059 treatment enhanced ara-C-mediated apoptosis. Likewise, Wang et al. (31) demonstrated that H2O2- induced apoptosis was enhanced when ERK activation was inhibited by PD 98059 in HeLa cells.

ERK can phosphorylate a large number of both cytoplasmic and nuclear proteins (10). Of particular interest are transcription factors and other nuclear proteins that may be regulated by ERK, including Elk-1, c-Fos, ATF-2, c-Myc, NF-IL6, Ets-2, TAL-1, p53, and RNA polymerase II (10). Many of these nuclear proteins, because of their ability to modulate expression of other proteins, are potential candidates for critical factors involved in the cellular response to cisplatin. For example, p53 modulates a number of cellular responses to genotoxic stress (32). In vitro studies have demonstrated that ERK has the capability of phosphorylating p53 at two N-terminal sites, throne 73 and 83; however, the in vivo physiological consequences of the potential phosphorylation of p53 by ERK has not been determined (33). Another potential target of ERK, the nuclear protein c-fos, previously had been shown to have increased expression in cisplatin-resistant cell lines and in tumor cells from patients treated with cisplatin (34, 35). The c-fos protein, in turn, can modulate the expression of dTMP synthase, topoisomerase I, and metallothionein, genes involved in DNA repair (36, 37). The other potential targets of the ERK pathway that may participate in modulation of the cellular response to cisplatin include p21WAF and nuclear factor κB.

Additional potential downstream effects of ERK activation that promote cell survival in response to cisplatin are currently under investigation in our laboratory. A better understanding of the signal transduction pathways that modulate the cisplatin response may be beneficial in the development of novel therapeutic approaches for improvement of cisplatin efficacy and circumvention of cisplatin resistance.

References
Inhibition of ERK Enhances Cisplatin Sensitivity


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