Arch Pharm Res Vol 29, No 2, 152-158, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Overexpression of p73 Enhances Cisplatin-Induced Apoptosis in HeLa Cells

Keun-Cheol Kim, Chul-Soo Jung[1], and Kyung-Hee Choi[1]

Division of Life Sciences, College of Natural Sciences, Kangwon National University, Chuncheon 200-701, Korea and [1]Department of Life Science, College of Natural Sciences, Chung-Ang University, Seoul 156-756, Korea

(Received December 15, 2005)

To examine a possible synergistic role for p73 and cisplatin (*cis*-diamminedichloroplatinum II) in HeLa cells with a nonfunctional p53 protein, we established stable HeLa/p73 clones using a tetracycline inducible eukaryotic expression vector. The HeLa/p73 clones were not characterized by changes in growth or morphology. Cell death analysis, however, indicated a greater sensitivity to cisplatin in the p73-overexpressed HeLa cells than determined for the non-induced HeLa cells. This increased sensitivity seems to affect an induction of a sub-G1 population as assessed from flow cytometry analysis. The increased sub-G1 population may, in turn, result from a reduction of cyclin D1 and B1 expression by cisplatin in the presence of p73. Hoechest staining indicated an increased number of dead cells in the p73-induced cells compared to the non-induced cells. Poly ADP-ribose polymerase (PARP) cleavage was shown to be distinct in the p73-overexpressed cells compared to non-induced cells, which suggests that p73 modulates the cisplatin-induced apoptosis. Therefore, a synergistic effect of p73 and cisplatin to induce apoptosis could lead to new treatment for some types of human cancers.

Key words: Cisplatin, p73, Drug sensitivity, Cell death, Apoptosis

INTRODUCTION

Treatments for cancer have relied on the use of various cytotoxic chemotherapeutic agents and radiation. Application of the therapeutic strategy of choice, however, has been limited by the side effects of these agents on normal cells. Genetic alterations of human tumors can prevent the access of therapeutic agents to their site of action. Newer strategies for cancer chemotherapy are based on synergistic modulation directed at the selective and increased induction of apoptosis to kill tumor cells (Hannun, 1997).

Cisplatin (*cis*-diamminedichloroplatinum II) is one of the most effective chemotherapeutic agents currently in use for the treatment of a broad range of primary solid tumors and acts through an apoptosis pathway (Lee *et al.*, 2001). Cisplatin destroys the solid tumor cells by inducing apoptosis through the formation of intrastrand cisplatin-DNA adducts, which ultimately results in cell-cycle arrest at G1, S, or G2/M (Vaisman *et al.*, 1997). The clinical effectiveness of cisplatin monotherapy, however, has been limited due to drug resistance (Hannun, 1997). Tumor cell resistance to cisplatin has been attributed to such factors as increased and effective DNA repair, increased drug tolerance, and increased intracellular glutathione levels (Poppenborg *et al.*, 1997). Drug resistance is a severe obstacle for the success of the cancer chemotherapies. Some biochemical modulators reportedly sensitize tumor cells to the effects of cisplatin and thus may play an important role in the modulation of cytotoxicity (Vaisman *et al.*, 1997). Several investigators have reported that caffeine and other methylxanthines can enhance the cyotoxic effects of cisplatin by the abrogation of G2 arrest induced by DNA damage (Husain *et al.*, 1998; Dubrez *et al.*, 2001). Moreover, bcl-2 antisense therapy has been reported to improve the results of chemotherapy for melanoma and the combination of IL-1 alpha and cisplatin reportedly act synergistically to affect apoptosis (Duan *et al.*, 2004; Kim *et al.*, 2004). One of the key modulators of apoptosis in malignancy is p53, which regulates cellular responses to different types of intracellular stresses such as DNA damaging agents (Feng *et al.*, 2000). Wild type p53 transduction in pancreatic tumor

Correspondence to: Keun-Cheol Kim, Division of Life Sciences, College of Natural Sciences, Kangwon National University, Chuncheon 200-701, Korea
Tel: 82-33-250-8532, Fax: 82-33-251-3990
E-mail: kckim@kangwon.ac.kr

cells reportedly increased the cytotoxicity of genotoxic drugs (Cascallo *et al.*, 2000). Moreover, it has been reported that p53 is important in the response of human colorectal tumor cells to different chemotherapeutic agents, which further suggests that the combination of p53 and cisplatin could be a very promising new therapy (Bunz *et al.*, 1999).

Within the p53 family of factors, p73 activates transcription of p53-responsive genes e.g. p21 or bax and inhibit cell growth in a p53-like manner by inducing apoptosis in p53 null Saos-2 cells (Jost *et al.*, 1997; Kaghad *et al.*, 1997). However, a number of differences between p73 and p53 have been reported. Tyrosine kinase c-abl stabilized p73 at the posttranslation level in cisplatin-induced DNA damaged cells (Gong *et al.*, 1999; Yuan *et al.*, 1999). Some regulatory proteins including c-abl modulate nuclear translocation of p73 in cells with nonfunctional p53 (Kim *et al.*, 2001; Puig *et al.*, 2003; Sphyris *et al.*, 2004). These previous reports suggest that the apoptotic activity of p73 is potentiated by distinct pathway in response to DNA damage. It is still unclear, however, how p73 can regulate cell growth in some tumor cells.

In this study, we addressed the hypothesis that p73 plays a synergistic role in cisplatin-induced apoptosis in human cancer cells with nonfunctional p53 proteins. To test our hypothesis, we constructed a HeLa/p73 cell line using a tetracycline-inducible system in human cervical carcinoma HeLa cells. Previous reports had indicated that the HeLa cell has a nonfunctional p53 protein due to binding with the HPV E6 protein (Kessis *et al.*, 1993; Minagawa *et al.*, 1999; Wei, 2005). Cellular responsiveness to cisplatin was examined using various cytotoxic assays in both non-induced HeLa cells and the p73-induced HeLa cells.

MATERIALS AND METHODS

Cell culture and transfection

The HeLa cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD). HeLa cells were maintained as a monolayer culture in DMEM (GIBCO-BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT). Full-length p73 (gift from Dr. Kaghad, Sanofi Recherche, France) was cloned into the Xba1/EcoR1 sites of the expression vector pUHD10-3. HeLa cells were transfected with the tetracycline-inducible plasmid by lipofectamine (GIBCO-BRL, Gaithersburg, MD). Cells were then grown in complete medium containing 100 mg/mL G418 (Stratagene, La Jolla, CA). Colonies were isolated and expanded into cell clones at the end of four weeks.

Cytotoxicity assay

Cytotoxicity to cisplatin was determined by the sulforhodamine B (SRB) assay. Cells were plated in triplicate and the assay carried out in standard 96 well plates. Tetracycline (2 mg/mL) was added to the cells for 12 h and treated for additional 12 h with various concentrations of cisplatin (dissolved in distilled water). The plates were fixed with 10% trichloroacetic acid (TCA) and stained with SRB. After rinsing the plate with 1% acetic acid, it was solubilized with 10 mM Tris buffer (pH 10.5). Cell viability was analyzed with an ELISA reader (Bio-Rad, Hercules, CA) at OD_{570}. To examine DNA fragmentation, cells were extracted with DNA extraction buffer (10 mM Tris-HCl (pH 8.0), 100 mM NaCl, 25 mM EDTA (pH 8.0), 0.5% SDS, Proteinase K, 0.1 mg/mL). Genomic DNA was obtained by phenol/chloroform extraction, precipitated with absolute ethanol, separated on 1.5% agarose gel, and visualized with ethidium bromide staining.

Flow cytometry analysis

Cell populations (1×10^5) were cultured in 60 mm dishes. After the induction of p73 with tetracycline, cisplatin was added and the treatment continued for 24 h. Cells were then harvested and stained with propidium iodide. Cell cycle distribution was analyzed according to the protocols of Becton Dickinson (Becton Dickinson, Mountain View, CA).

Hoechest staining

Hoechest staining was performed according to a previous report (Kang *et al.*, 1999). Briefly, cells were fixed with 3.7% formaldehyde and stained by Hoechest 33258 (Sigma, St.Louis, MO) for 30 min. A minimum of 500 cells were counted and scored for the incidence of apoptotic morphology as an unbiased manner.

Western blot analysis

Whole cell lysates were prepared in TNN buffer (40 mM Tris (pH 8.0), 120 mM NaCl, 0.1% NP-40). Protein samples were separated by 7.5-12% SDS-PAGE, the gels blotted to nitrocellulose filters, and then hybridized with anti-p21, anti-bax, anti-cyclin D1, anti-cyclin B1, and anti-PARP (Santa Cruz, San Diego, CA). Anti-p73 polyclonal rabbit serum was prepared against the N-terminal fragment of p73 and processed by affinity chromatography purification.

Statistical analysis

Data are presented in experimental graphs as the mean ± standard deviation (SD). Statistical analyses were performed using student's *t*-test. Differences were considered significant at $p < 0.05$.

RESULTS

Isolation of p73-overexpressed HeLa cells

After the transfection of the tetracycline inducible vector containing p73 cDNA into the HeLa cells, we isolated the p73-overexpressed clones and designated them as HeLa/p73. The expression of p73 was increased in the presence of tetracycline when compared to the non-tetracycline exposed HeLa cells (Fig. 1A). The mechanism of action of p73 involves the induction of target genes (Kaghad *et al.*, 1997; Kang *et al.*, 2002). As expected, the p73 overexpression also increased p21 and bax, which are well-known target proteins for p73. The growth and the morphology of the HeLa cells and the p73-overexpressed HeLa cells, however, was not changed (data not shown). These results suggest that the expression of p73 is regulated by tetracycline in the HeLa/p73 cells.

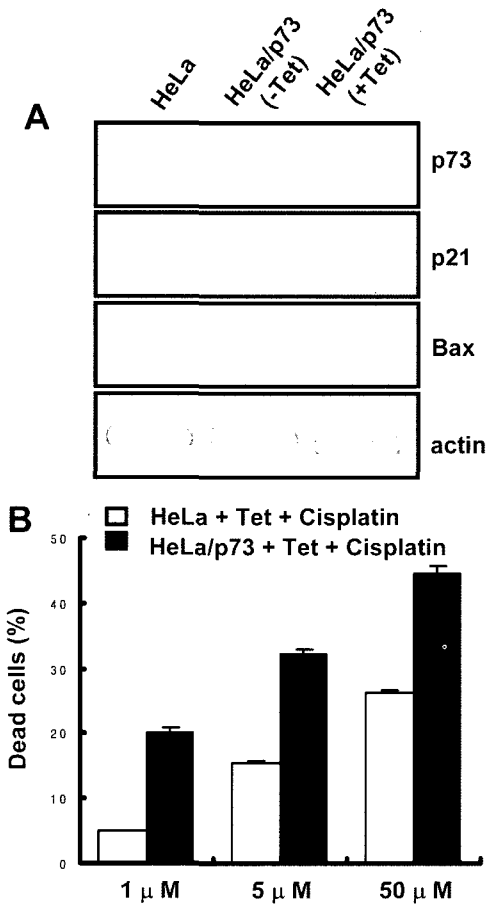

Fig. 1. Tetracycline-inducible p73 expression in HeLa cells. (A). HeLa and HeLa/p73 cells were cultured in the presence (2 mg/mL) or absence of tetracycline (Tet). Western blot analysis was performed after loading 30 mg of protein lysates onto 10% SDS-PAGE (B). Cells were treated by indicated concentrations of cisplatin for 24 h after p73 was induced with tetracycline for 12 h. SRB assay was performed as described in Materials and Methods. This graph was obtained from triplicate experiments.

Overexpression of p73 enhances cisplatin-induced apoptosis in HeLa cells

To examine a possible synergistic role for p73 on cisplatin-induced apoptosis, we treated various concentrations of cisplatin for 24 h in both HeLa cells and p73-overexpressed HeLa cells. The apoptotic effect of cisplatin varied as the concentration increased in both HeLa and p73-overexpressed HeLa cells. Since HeLa cells are without p53 activity, they could be mildly resistant to cisplatin-induced apoptosis (Minagawa *et al.*, 1999). The overexpression of p73 resulted in fold induction of the apoptotic effect to cisplatin (Fig. 1B). A synergistic effect of p73 on cisplatin-induced apoptosis was also confirmed by an internucleosomal DNA fragmentation assay, which yielded the similar results (Fig. 2). We suggest, therefore, that p73 promotes the cisplatin-induced apoptosis in HeLa cells.

Enhanced effects of p73 was not correlated with the arrest of G1 or G2/M

It has been proposed that cell death might be correlated with the arrest of G1 or G2/M after anticancer drug treatment in tumor cell lines (Vaisman *et al.*, 1997; Qin and Ng, 2001; Sphyris *et al.*, 2004). To study the mechanism for p73 sensitization to cisplatin-induced apoptosis, we per-

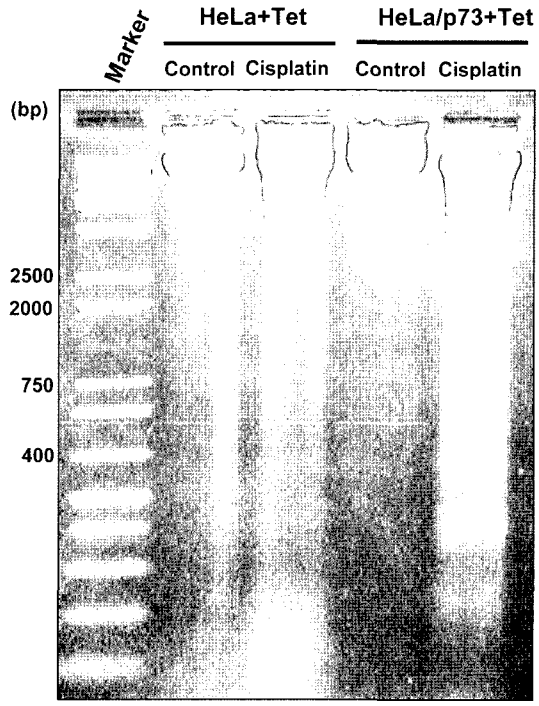

Fig. 2. DNA fragmentation in HeLa and p73-overexpressed HeLa cells. p73 was induced with 2 mg/mL tetracycline for 12 h. Genomic DNA was extracted at 24 h after 50 mM cisplatin treatment, resolved in 1.5% agarose gel electrophoresis, and visualized with ethidium bromide staining.

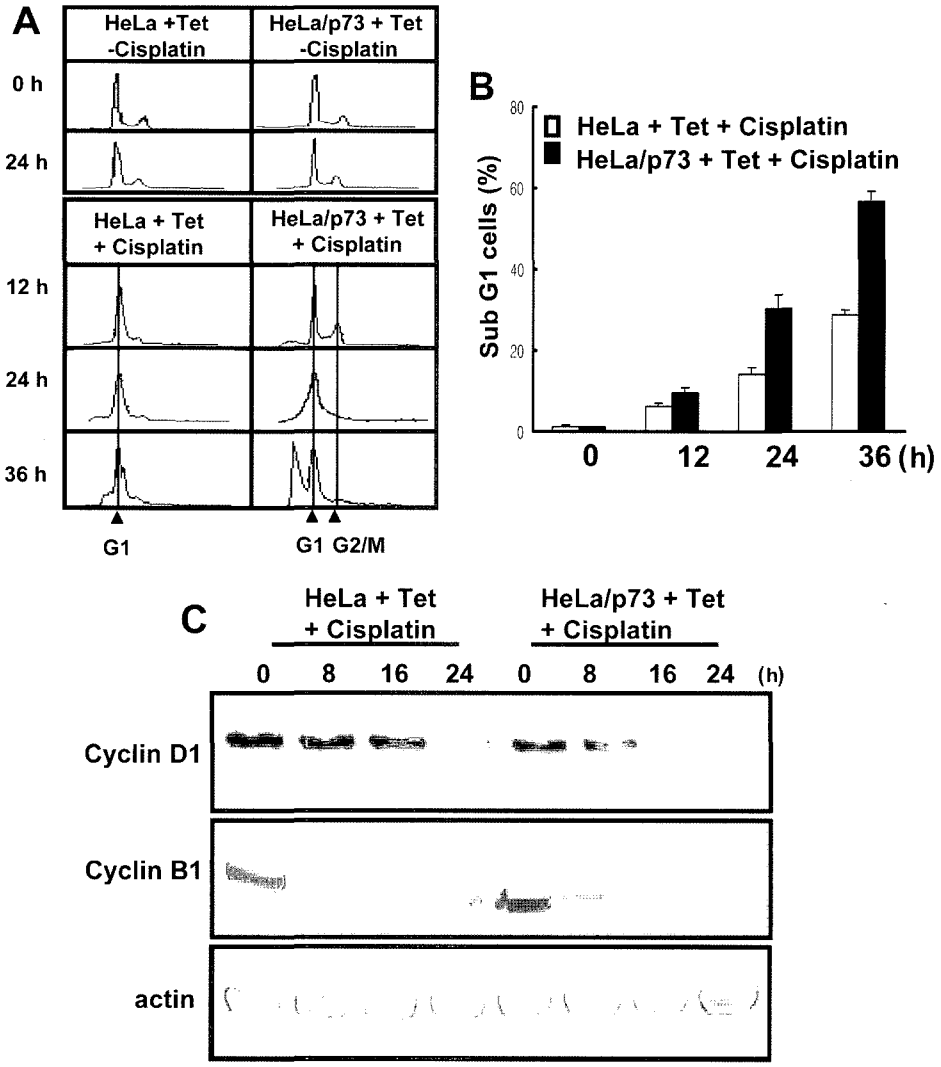

Fig. 3. Cell-cycle analysis and G1 or G2/M cyclins expression in HeLa and p73-overexpressed HeLa cells. (A) p73 was induced with 2 mg/mL tetracycline for 12 h. Fifty mM of cisplatin was added to the cells for the indicated times. Cells were harvested and incubated in propidium iodide/RNase A for 30 min. Cell-cycle analysis was performed according to the manufacturer's protocol (Beckton Dickinson, U.S.A.). (B) Sub-G1 population was calculated by mean ± SD. (C) It was performed for p73 induction and cisplatin treatment. Cells were harvested and Western blot analysis was performed using the indicated antibodies. Actin antibody was used as a loading control

formed a flow cytometry analysis following cisplatin treatment of HeLa and HeLa/p73 cells. Cell cycle analysis demonstrated that there was no significant difference on cell cycle distribution in HeLa or p73-induced HeLa cells in the absence of cisplatin (Fig. 3A, upper). Cisplatin treatment, however, accelerated the induction of sub-G1 cells in p73-induced HeLa cells compared to non-induced HeLa cells (Fig. 3A, B). We also analyzed the cyclin D1 and B1 expression in the combination of p73 and cisplatin. As shown in Fig. 3C, cyclin D1 and B1 expression were dramatically decreased by cisplatin in the presence of p73, indicating that cisplatin induces cell death without cell cycle arrest in HeLa cells. It might be assumed from these results that p73 sensitizes cisplatin-induced apoptosis in a cell-cycle independent manner by modulating cell-cycle regulatory proteins in HeLa cells.

Overexpression of p73 modulates cisplatin-induced apoptosis

Flow cytometry analysis data was consistent with the interpretation that p73 expression modulates cisplatin-induced apoptosis in the HeLa cells. To further investigate this indication, we performed hoechest 33258 staining and poly ADP-ribose polymerase-1 (PARP) cleavage using a 50 mM cisplatin treatment in both the HeLa and the p73-overexpressed HeLa cells. The morphology of apoptotic cells were examined and photographed using fluorescence microscopy. As shown in Fig. 4, a cisplatin-induced apoptotic morphologic change was clearly observed in the p73-overexpressed HeLa cells when compared to

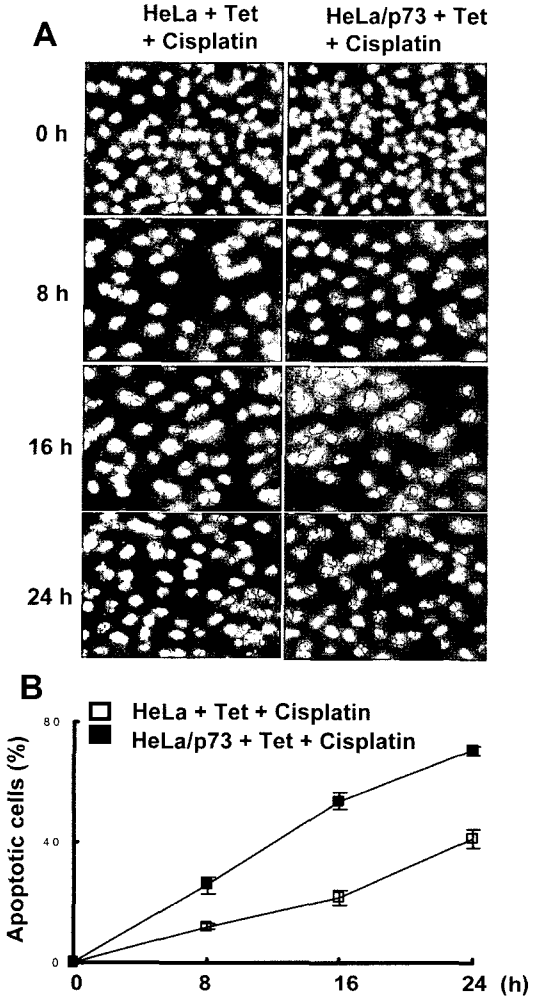

Fig. 4. Counting of dead cells by Hoechst staining. (A) Cells were seeded and stained with hoechest 33258 at the indicated time points and photographed using a Nicon Eclipse TE 300 microscope (100X). (B) The percentages of apoptotic cells were determined. Apoptotic cells were counted at five areas without any bias. Experimental data is expressed as the mean ± SD.

HeLa cells. A difference in sensitivity to cisplatin was also evident when the PARP cleavage effects were measured using western blot analysis. PARP cleavage was found at 8 h after the start of cisplatin treatment in the p73-overexpressed HeLa cells, whereas only at 16 h in HeLa cells (Fig. 5). Thus, these results suggest that p73 accelerates the cisplatin-induced apoptosis.

DISCUSSION

By understanding the different pathways that contribute to cell death, one or more of these pathways could be targeted to induce apoptosis. Cisplatin is one of the chemotherapeutic agents that induces apoptosis in human cancers. Resistance to cisplatin in various cancers, specifically solid tumors, however, is an ongoing therapeutic challenge. Therefore, understanding the expression of cell cycle regulatory molecules is a worthwhile goal to increase drug efficacy (Hannun, 1997; Qin and Ng, 2001; Sphyris *et al.*, 2004). As a tumor suppressor p53 homolog, the p73 protein is a target of c-abl tyrosine kinase in response to cisplatin or radiation based damage to DNA (Agami *et al.*, 1999; Yuan *et al.*, 1999). A role for p73 in the cellular responses to DNA damage, however, has not been investigated.

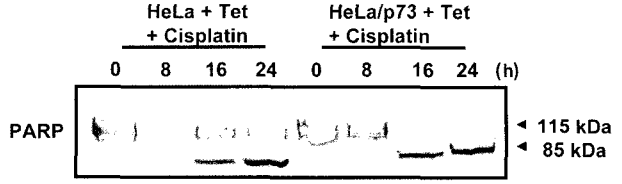

Fig. 5. PARP cleavage in HeLa and p73-overexpressed HeLa cells. After the induction of p73, the cells were treated with 50 mM cisplatin for the indicated time periods. Western blot analysis was performed using anti-PARP antibody.

In the present study, we asked whether p73 has a possible role in modulating cisplatin-induced apoptosis. Our studies used the HeLa cervical carcinoma cell, which has nonfunctional p53 due to its binding with the human papillomavirus (HPV) E6 protein (Kessis *et al.*, 1993). The binding of HPV E6 protein to E6-associated protein (E6AP), a cellular ubiquitin-protein ligase, enables E6AP to ubiquitinate p53, which leads to p53 degradation in HeLa cells (Wei, 2005). HeLa cells show a poor response to DNA damaging agents, including cisplatin (Minagawa *et al.*, 1999). In our studies, we did not detect any apoptosis-like characteristics in the HeLa/p73 clones as determined by trypan blue exclusion, although p73 were highly expressed by treatment of tetracycline. This may explain that p73, like p53 or bax, induces apoptosis in a cell context-dependent manner (Fang *et al.*, 1999). Our data show that cisplatin in the presence of p73, however, was associated with accelerating apoptosis in HeLa cells, which suggests that p73 is an apoptosis-related tumor suppressor protein.

The induction of drug sensitivity by a tumor suppressor protein may be mediated by cell-cycle arrest in tumor cell lines. This is especially the case with the expression of G1 phase regulatory proteins which have been shown to modulate sensitivity to cisplatin in various cancer cells. Thus, for p21 and cisplatin, the p21 induction appears to correlate with increased apoptosis after G1 arrest has been induced by anticancer drugs in hepatocellular carcinoma cells (Qin and Ng, 2001) and primary pancreatic cells (Sphyris *et al.*, 2004). Also, the expression of p27 was strongly associated with enhanced chemosensitivity in acute myeloid leukemia cells (Radosevic *et al.*, 2001).

The combination of cisplatin and wild type p53, however, elicited induction of a sub-G1 population in human cancer cells (Cascallo *et al.*, 2000; Dunkern *et al.*, 2001). In this report, we have identified significant increases in a sub-G1 population when p73 is overexpressed. In the presence of p73, cisplatin treatment severely decreased the expression of both cyclin D1 and B1, which are cell-cycle regulatory proteins for the G1 and G2/M phases, respectively. This result may explain why the combination of the p73 protein and cisplatin accelerate cell death without cell cycle arrest in the HeLa cells. The hoechest staining and PARP cleavage studies consistently indicated greater dead cell populations in the p73-overexpressed HeLa cells compared to the HeLa cells. Thus, our results suggest that the combination of p73 and cisplatin depends upon the induction of sub-G1 cells.

In this paper, we have focused on a synergistic therapeutic strategy which may confer additional antitumor efficacy for chemotherapeutic agents. Our work tests the hypothesis that intracellular modulators may induce sensitivity to chemotherapeutic agents. The synergistic mechanism involving a p73- cisplatin-induced apoptosis seems to be based upon accelerating cell death. Thus, the combination of p73 and cisplatin may be the basis for new therapies for certain types of human cancers.

REFERENCES

Agami, R., Blandino, G., Oren, M., and Shaul, Y., Interaction of c-Abl and p73a and their collaboration to induce apoptosis. *Nature,* 399, 809-813 (1999).

Bunz, F., Hwang, P.M., Torrance, C., Waldman, T., Zhang, Y., Dillehay, L., Williams, J., Lengauer, C., Kinzler, K., and Vogelstein, B., Disruption of p53 in human cancer cells alters the responses to therapeutic agents. *J. Clin. Invest.,* 104, 263-269 (1999).

Cascallo, M., Calbo, J., Gelpi, J. L., and Mazo, A., Modulation of drug cytotoxicity by reintroduction of wild type p53 gene (Ad5CMV-p53) in human pancreatic cancer. *Cancer Gene Ther.,* 7, 545-556 (2000).

Duan, L., Aoyagi, M., Tamaki, M., Yoshino, Y., Morimoto, T., Wakimoto, H., Nagasaka, Y., Hirakawa, K., Ohno, K., and Yamamoto, K., Impairment of both apoptotic and cytoprotective signalings in glioma cells resistant to the combined use of cisplatin and tumor necrosis factor alpha. *Clin. Cancer Res.,* 10, 234-243 (2004).

Dubrez, L., Coll, J. L., Hurbin, A., Solary, E., and Favrot, M. C., Caffeine sensitizes human H358 cell line to p53-mediated apoptosis by inducing mitochondrial translocation and conformational change of BAX protein. *J. Biol. Chem.,* 276, 38980-38987 (2001).

Dunkern, T. R., Fritz, G., and Kaina, B., Cisplatin-induced apoptosis in 43-3B and 27-1 cells defective in nucleotide excision repair. *Mutat. Res.,* 486, 249-258 (2001).

Fang, L., Igarashi, M., Leung, J., Sugrue, M., Lee, S., and Aaronson, S., p21 Waf2/Cip1/Sdi1 induces permanent growth arrest with markers of replicative senescence in tumor cells lacking functional p53. *Oncogene,* 18, 2789-2797 (1999).

Feng, L., Achanta, G., Pelicano, H., Zhang, W., Plunkett, W., and Huang, P., Role of p53 in cellular response to anticancer nucleoside analog-induced DNA damage. *Int. J. Mol. Med.,* 5, 597-604 (2000).

Gong, J. G., Costanzo, A., Yang, H.Q., Melino, G., Kaelin, W. G. Jr., Levrero, M., and Wang, J.Y., The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. *Nature,* 399, 806-809 (1999).

Hannun, Y. A., Apoptosis and the dilemma of cancer chemotherapy. *Blood,* 89, 1845-1853 (1997).

Husain, A., Rosales, N., Schwartz, G. K., and Spriggs, D. R., Lisophylline sensitizes p53 mutant human ovarian carcinima cells to the cytotoxic effects of *cis*-diamminedichloroplatinum (II). *Gynecol. oncol.,* 70, 17-22 (1998).

Jost, C. A., Marin, M. C., and Kaelin, W. G. Jr., p73 is a human p53-related protein that can induce apoptosis. *Nature,* 389, 191-194 (1997).

Kaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J. C., Valent, A., Minty, A., Chalon, P., Lelias, J. M., Dumont, X., Ferrara, P., McKeon, F., and Caput, D., Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell,* 90, 809-819 (1997).

Kang, K. H., Lee, J. H., Kim, K. C., Ham, S. W., Kim, M. Y., and Choi, K. H., Induction of p73beta by a naphthoquinone analog is mediated by E2F-1 and triggers apoptosis in HeLa cells. *FEBS Lett.,* 522, 161-167 (2002).

Kang, K. H., Kim, W. H., and Choi, K. H., p21 promotes ceramide-induced apoptosis and antagonizes the antideath effect of bcl-2 in human hepatocarcinoma cells. *Exp. Cell Res.,* 253, 403-412 (1999).

Kessis, T. D., Slebos, R. J., Nelson, W. G., Kastan, M. B., Plunkett, B. S., Han, S. M., Lorincz, A. T., Hedrick, L., and Cho, K. R., Human papillomavirus 16 E6 expression disrupts the p53-mediated cellular response to DNA damage. *Proc. Natl. Acad. Sci. U.S.A.,* 90, 3988-3992 (1993).

Kim, K. C., Kim, T. S., Kang, K. H., and Choi, K. H., Amphiphysin IIb-1, a novel splicing variant of amphiphysin II, regulates p73beta function through protein-protein interactions. *Oncogene,* 20, 6689-6699 (2001).

Kim, R., Emi, M., Tanabe, K., and Toge, T., Therapeutic potential of antisense Bcl-2 as a chemosensitizer for cancer therapy. *Cancer,* 101, 2491-2502 (2004).

Lee, R. H., Song, J. M., Park, M. Y., Kang, S. K., Kim, Y. K., and Jung, J. S., Cisplatin-induced apoptosis by translocation of endogenous Bax in mouse collecting duct cells. *Biochem. Pharmacol.*, 62, 1013-1023 (2001).

Minagawa, Y., Kigawa, J., Itamochi, H., Kanamori, Y., Shimada, M., Takahashi, M., and Terakawa, N., Cisplatin-resistant HeLa cells are resistant to apoptosis via p53-dependent and –independent pathways. *Jpn. J. Cancer Res.*, 90, 1373-1379 (1999).

Poppenborg, H., Munstermann, G., Knupfer, M. M., Hotfilder, M., and Wolff, J. E. A., C6 cells cross-resistant to cisplatin and radiation. *Anticancer Res.*, 17, 2073-2078 (1997).

Puig, P., Capodieci, P., Drobnjak, M., Verbel, D., Prives, C., Cordon-Cardo, C., and Di Como, C. J., p73 Expression in human normal and tumor tissues : loss of p73α expression is associated with tumor progression in bladder cancer. *Clin. Cancer Res.*, 9, 5642-5651 (2003).

Qin, L. F. and Ng, I. O., Exogenous expression of p21(WAF1/CIP1) exerts cell growth inhibition and enhances sensitivity to cisplatin in hepatoma cells. *Cancer Lett.*, 172, 7-15 (2001).

Radosevic, N., Delmer, A., Tang, R., Marie, J. P., and Ajchenbaum-Cymbalista, F., Cell cycle regulatory protein expression in fresh acute myeloid leukemia cells and after drug exposure. *Leukemia,* 15, 559-566 (2001).

Sphyris, N., Morris, R. G., and Harrison, D. J., Induction of p21 and nuclear accumulation of TAp73alpha and c-abl during apoptosis of cisplatin-treated primary pancreatic acinar cells. *Int. J. Oncol.*, 25, 1661-1670 (2004).

Vaisman, A., Varchenko, M., Said, I., and Chaney, S. G., Cell cycle changes associated with formation of Pt-DNA adducts in human ovarian carcinoma cells with different cisplatin sensitivity. *Cytometry,* 27, 54-64 (1997).

Wei, Q., Pitx2a binds to human papillomavirus type 18 E6 protein and inhibits E6-mediated P53 degradation in HeLa cells. *J. Biol. Chem.*, 280, 37790-37797 (2005).

Yuan, Z. M., Shioya, H., Ishiko, T., Sun, X., Gu, J., Huang, Y. Y., Lu, H., Kharbanda, S., Weichselbaum, R., and Kufe, D., p73 is regulated by tyrosine kinase c-abl in the apoptotic response to DNA damage. *Nature,* 399, 814-817 (1999).