Induction of p53-mediated apoptosis and recovery of chemosensitivity through p53 transduction in human glioblastoma cells by cisplatin

CHANG-MIN PARK[1*], MYUNG-JIN PARK[1*], HEE-JIN KWAK[1], SANG-IK MOON[1], DOO-HYUN YOO[1], HYUNG-CHAHN LEE[1], IN-CHUL PARK[1], CHANG HUN RHEE[1,2] and SEOK-IL HONG[1,3]

[1]Laboratory of Functional Genomics, [2]Department of Neurosurgery, and [3]Laboratory Medicine and Clinical Pathology, Korea Institute of Radiological and Medical Sciences, Seoul, Korea

Received June 3, 2005; Accepted July 13, 2005

Abstract. Cisplatin is a DNA-damaging chemotherapeutic drug that may have a role in the adjuvant chemotherapy of several solid tumors, such as malignant glioblastoma, and the status of p53 tumor suppressor protein is a critical determinant of cisplatin chemosensitivity. In the present study, we showed the relationship of p53 status and chemosensitivity of cisplatin between two human malignant glioblastoma cell lines, A172 and T98G, harboring wild-type and mutant-type p53, respectively. Cisplatin was found to be more cytotoxic to A172 than T98G cells in a time- and concentration-dependent manner. Cisplatin-induced cytotoxicity manifested as apoptosis, characterized by genomic DNA fragmentation, nuclear condensation and an increase in sub-G1 population. Cisplatin induced the accumulation of p53 and p21 proteins in A172 cells, but not in T98G cells. The introduction of the adenovirus-mediated wild-type *p53* gene into T98G cells resulted in the decrease of viability as well as the increase in sub-G1 population with p53 accumulation, activation of caspase-3 protease and release of cytochrome c from the mitochondria. These data strongly suggest that the expression of p53 is essential for the cytotoxic effect of cisplatin in human malignant glioblastoma cells, A172 and T98G, and the introduction of apoptotic signal molecules, such as p53, will be beneficial to achieve chemosensitivity in malignant glioma.

Introduction

Gliomas are the common primary brain tumor of the adult human central nervous system. Despite innumerable efforts to develop more effective clinical treatment strategies, the median survival time for patients with WHO grade 4 glioma, glioblastoma multiforme, remains at approximately 1 year (1-4). An important factor contributing to this prognosis is the relative chemoresistance of many gliomas to chemotherapy in general (5-7). Nonetheless, chemotherapy is the most widely used modality in the management of gliomas (8). The chemoresistance of tumor cells to anti-cancer drugs is frequently associated with defects in the signaling pathways leading to apoptosis. The product of the *p53* tumor suppressor gene has been shown to be responsible for mediating apoptosis after DNA damage in several cell types (9), and the loss of wild-type p53 can render cells resistant to chemotherapeutic agents or radiation (10,11). Actually, the most common genetic alteration in human cancer, including brain cancer, involves the p53 tumor suppressor gene, and mutation or inactivation of p53 is observed in approximately 50% of human cancers.

The p53 tumor suppressor gene is the central integrator of the cellular response to DNA damage, oncogenic transformation, and growth factor withdrawal (12-14). p53 plays a central role in the regulation of DNA-damage-induced apoptosis by up-regulating the transcription of genes encoding Bax, Noxa and p53AIPI, which participate in the intrinsic apoptotic pathway (15-17). The release of cytochrome c from mitochondria is a central event in the death receptor-independent intrinsic pathway of apoptosis (18,19). Cytochrome c together with dATP and Apaf-1 facilitates the activation of caspase-9 protease, which further activates procaspase-3 protease (20,21). Caspase-3 protease cleaves their substrates, including PARP, to lead to apoptotic cell death. It has also been reported that the activation of caspase-3 protease in drug-induced apoptosis modulates the activity of caspase-8 protease in a CD95/Fas receptor-independent manner (22-25). Several studies established the involvement of caspase activation in p53-mediated cell death (26). Recently, the requirement of Apaf-1 or caspase-9 protease for the p53-dependent apoptosis of oncogene-transformed murine embryonic fibroblasts has conclusively been demonstrated (27). In addition to involvement in the regulation of apoptosis, p53 also plays a role in DNA-damage repair processes by transcriptional regulation of several downstream mediators that are involved in cell-cycle regulation. p21/WAF1/CIP1 is

Correspondence to: Dr Seok-Il Hong, Laboratory of Functional Genomics, Laboratory Medicine and Clinical Pathology, Korea Institute of Radiological and Medical Sciences, 215-4 Nowon-Ku, Gongneung-Dong, Seoul 139-706, Korea
E-mail: hongsicp@kcch.re.kr

[*]Contributed equally

Key words: cisplatin, glioblastoma, chemosensitivity, apoptosis, p53

well known as one of the downstream mediators of p53, inhibiting cyclin-dependent kinases (28-30). The cell-cycle regulatory and DNA repair functions of p53 are largely executed by transactivation of p53-response genes, including p21/WAF1/CIP1, thus relying on the ability of p53 to act as a sequence-specific transcription factor. DNA-damaging agents, such as ionizing radiation, ultraviolet light and chemotherapeutic agents, have previously been shown to induce p53-dependent and -independent p21/WAF1/CIP1 expression in various cancer cell types (31-33).

Cisplatin is a platinum-based chemotherapeutic agent used for the treatment of many malignancies, including glioma (34). The cytotoxicity of cisplatin is thought to be due to the formation of intrastrand and interstrand cross-links in DNA, possibly inducing cell-cycle arrest and apoptosis (35). Since cisplatin-induced DNA adducts have been shown to be repaired predominantly by nucleotide excision repair, the cytotoxicity of cisplatin may be influenced by the status of functional p53 in a cell through its effects on the cell cycle or apoptosis.

In this study, we compared the chemosensitivity of human glioma cell lines, A172 and T98G, to cisplatin which have wild-type and mutant-type p53, respectively, and found that cisplatin was chemosensitive to A172 cells but chemoresistant to T98G cells. The introduction of wild-type p53 to T98G cells resulted in a marked enhancement of cisplatin sensitivity by induction of apoptotic cell death via p53-dependent p21 expression, activation of caspase-3 and -9 proteases, and cytocrome c release.

Materials and methods

Reagents and cell culture. Cell culture supplies were purchased from Life Technologies, Inc. (Carlsbad, CA), and cisplatin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and other chemicals were purchased from Sigma (St. Louis, MO). The Wizard Genomic DNA purification kit was obtained from Promega (Madison, WI), and substrates for caspases were obtained from Calbiochem (La Jolla, CA). Antibodies for PARP, p53 and p21 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and anti-cytochrome c antibody was purchased from PharMingen (San Diego, CA). Enhanced chemiluminescence (ECL) reagent and donkey anti-rabbit IgG conjugated with horseradish peroxidase were purchased from Amersham (Buckinghamsire, UK). A172 and T98G cells were grown in DMEM medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), penicillin G (100 IU/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM) at 37˚C in a humidified atmosphere containing 5% CO_2 and 95% air.

Cell viability assay. Cells ($1x10^5$ cells/well) were seeded in 10% FBS/DMEM medium and were treated with cisplatin for indicated times. Measurement of mitochondrial activity to form purple formazan by MTT was used to assess the viability of cells following cisplatin treatment: MTT (0.5 mg/ml), one tenth of the original culture volume, was added to each culture and incubated for 3 h at 37˚C, in 5% CO_2. The purple formazan formed by viable cells was dissolved by the addition of DMSO, and absorbance at 540 nm was measured by using microplate reader (Titertek Multiskan, Flow Laboratories, USA).

DNA extraction and electrophoresis. The ladder pattern of a DNA strand break was analyzed by agarose gel electrophoresis. DNA from A172 and T98G cells was isolated by the Wizard Genomic DNA purification kit (Promega). After ethanol precipitation, 5 μg of DNA was subjected to electrophoresis on 1.5% agarose for 3 h at 50 V and visualized under UV light after staining with ethidium bromide.

Western blot analysis. To measure p53, p21, caspase-9, caspase-3-like protease and PARP cleavage, A172 and T98G cells were stimulated with cisplatin, harvested and washed with PBS, pH 7.4. The cell pellet was resuspended in a lysis buffer containing 0.5% Triton X-100, 25 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 μg/ml aprotinin. An equal amount of total protein, quantified by the Bicinchroninic acid kit (Sigma), was separated on SDS-PAGE and transferred onto nitrocellulose membrane. After blocking with 5% skim milk in TBS-T (25 mM Tris base, pH 7.6, 138 mM NaCl and 0.05% Tween-20), the membrane was incubated with anti-caspase-3 protease and anti-PARP antibodies (Santa Cruz) at room temperature for 2 h. Immunoblot was visualized by the ECL detection system (Amersham).

Preparation of cytosolic and membrane fractions. Cytosolic and membrane fractions were prepared according to the method previously reported (36) with some modification. In brief, cells were harvested, washed with ice-cold buffer A (20 mM HEPES pH 7.5, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, and 1 mM DTT), and then incubated with 100 μl of buffer A on ice for 30 min. Then, cells were disrupted by 20 passages through a 26-gauge needle. The disrupted cells were centrifuged at 750 x g for 10 min, and the supernatants obtained were centrifuged at 10,000 x g for 25 min. After centrifugation, the supernatants (cytosolic fractions) were frozen at -70˚C. The pellet containing mitochondria was washed with ice-cold buffer A and then resuspended in cell lysis buffer. The resuspended pellet was incubated on ice for 30 min and then centrifuged at 10,000 x g for 25 min. The supernatant was collected membrane fraction.

Fluorescent staining of nucleus. The nuclei of A172 and T98G cells were stained with chromatin dye, Hoechst 33258. Cells were fixed in PBS with 3.7% paraformaldehyde for 30 min at room temperature. The cells were then washed twice with PBS and incubated with 10 μM Hoechst 33258 in phosphate-buffered saline (PBS) at room temperature for 30 min. After three washes, the cells were observed under fluorescence microscope (Leica MPS 60, Germany) with excitation at 360 nm.

Measurement of caspase activity. To measure caspase activity, whole cell lysate was prepared in a lysis buffer (1% Triton X-100, 0.32 M sucrose, 5 mM EDTA, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 2 mM DTT, and 10 mM Tris-HCl, pH 8.0) on ice for 30 min and centrifuged at 20,000 x g for 15 min. Caspase-3-like protease activity in the supernatant

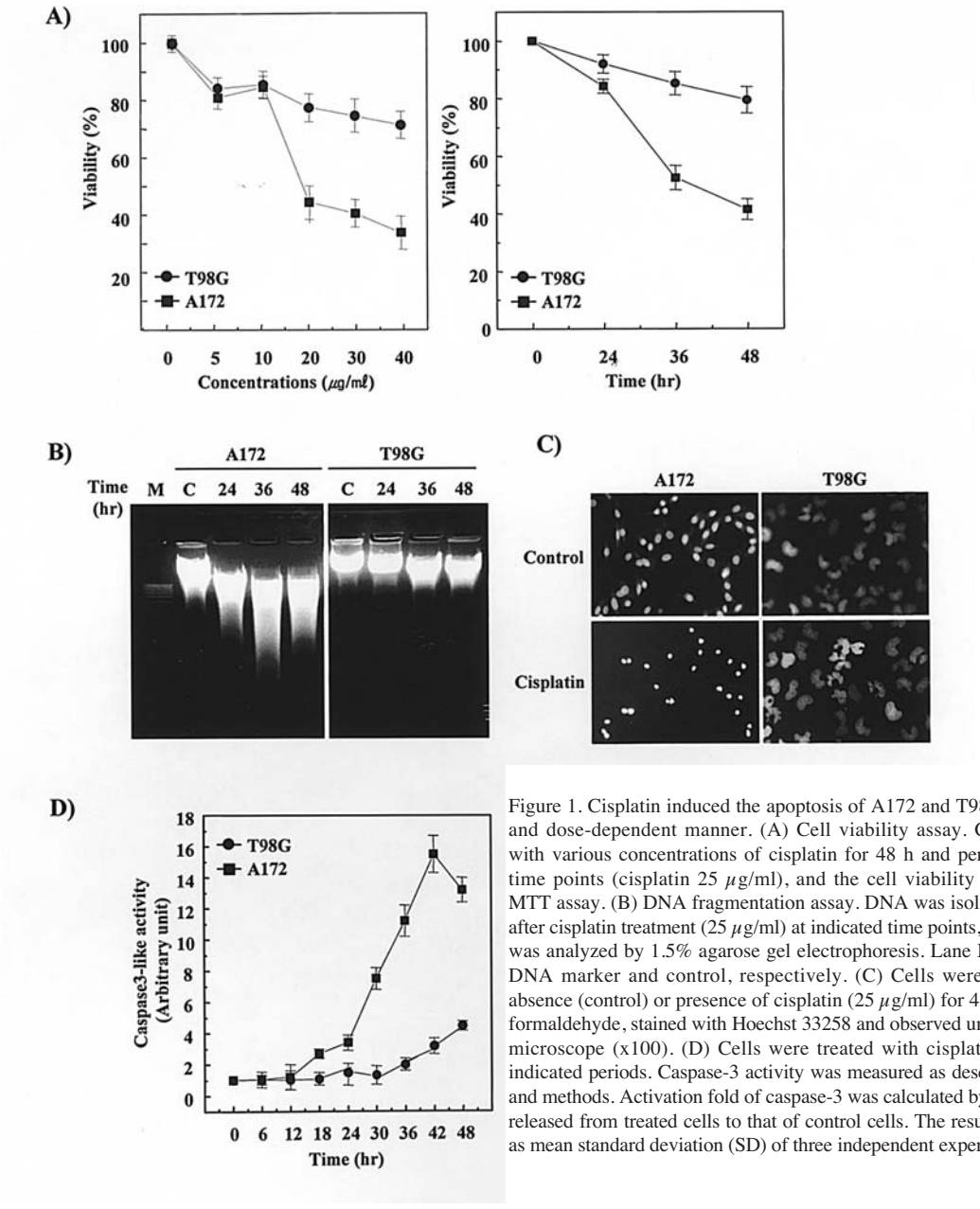

Figure 1. Cisplatin induced the apoptosis of A172 and T98G cells in a time- and dose-dependent manner. (A) Cell viability assay. Cells were treated with various concentrations of cisplatin for 48 h and periods for indicated time points (cisplatin 25 μg/ml), and the cell viability was measured by MTT assay. (B) DNA fragmentation assay. DNA was isolated from the cells after cisplatin treatment (25 μg/ml) at indicated time points, and fragmentation was analyzed by 1.5% agarose gel electrophoresis. Lane M and C represent DNA marker and control, respectively. (C) Cells were incubated in the absence (control) or presence of cisplatin (25 μg/ml) for 48 h, fixed with 4% formaldehyde, stained with Hoechst 33258 and observed under a fluorescence microscope (x100). (D) Cells were treated with cisplatin (25 μg/ml) for indicated periods. Caspase-3 activity was measured as described in Materials and methods. Activation fold of caspase-3 was calculated by the ratio of AMC released from treated cells to that of control cells. The results are represented as mean standard deviation (SD) of three independent experiments.

obtained above was measured by proteolytic cleavage of 100 μM 7-amino-4-methylcoumarin (AMC)-DEVD motif-specific peptide (Calbiochem) as a fluorogenic substrate for 1 h and AMC as a standard in an assay buffer (100 mM HEPES, 10% sucrose, 0.1% CHAPS, pH 7.5, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 2 mM DTT) using an excitation wavelength of 380 nm and emission wavelength of 460 nm. Similarly, cleavage of fluorogenic substrate, Ac-LEHD-7-amino-4-trifluoromethylcoumarin (AFC) (Calbiochem) by caspase-9 protease was measured by spectrofluorometer (Jasco FR-777, Germany) at 405/505 nm.

Flow cytometry. After an appropriate treatment, A172 and T98G cells (1x10^6 cells/ml) were harvested by centrifugation and washed with PBS. The cells were fixed with ice-cold 80% ethanol for 30 min, washed with PBS and then treated with 0.25 ml of 0.5% Triton X-100 solution containing 1 mg/ml propidium iodide for 30 min in the dark. Samples were run through a FACSter Vantage to count the number of cells (Becton Dickinson, San Jose, CA).

Statistical analysis. Each experiment was performed at least three times and all values are represented as means ± SD of triplicates. Statistical comparisons between groups were performed using one-way ANOVA, followed by the Student's t-test.

Results

Induction of apoptosis and cytotoxicity by cisplatin in human malignant glioblastoma A172 and T98G cells. Previous studies showed that anti-cancer drugs, such as cisplatin, induced apoptosis and that p53 status was thought to be an important factor in explaining the difference in cisplatin chemosensitivity among human glioblastoma cell lines (37,38). However, the molecular mechanisms of cisplatin-induced apoptosis remain unclear. In the present study, human malignant glioblastoma cells, A172 and T98G, were used to study the mechanism of p53 involvement in apoptosis induced by cisplatin. First, we treated the cells with cisplatin for an indicated time and dose and cell viability was measured by MTT assay. As shown in

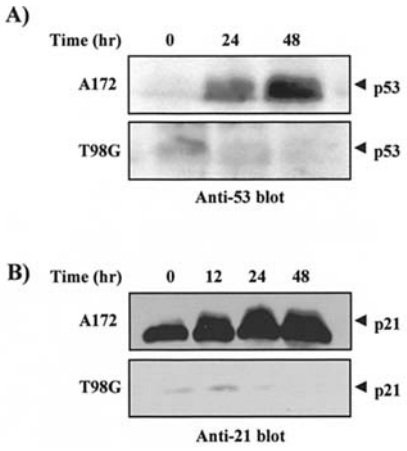

Figure 2. Cisplatin modulates the expression of p53 (A) and p21 proteins (B) in A172 but not in T98G cells. Cells were treated with cisplatin (25 μg/ml) for different time periods and lysate from the cells was used to measure p53 and p21 protein by Western blotting using anti-p53 and p21 antibodies.

Fig. 1A, cisplatin decreased the viability of A172 cells in a time- and dose-dependent manner, whereas there was only a marginal effect on T98G cells. Furthermore, cisplatin-induced cytotoxicity in A172 cells showed characteristics of apoptosis, manifested by genomic DNA fragmentation in agarose gel electrophoresis as well as nuclear condensation by Hoechst staining and activation of caspase-3-like protease (Fig. 1B-D) and caspase-9 (data not shown); however, this was to a lesser degree in T98G cells. These data clearly showed the difference in cisplatin sensitivity in the induction of apoptosis between the two cell lines.

Induction of p53 and p21/WAF1/CIP1 proteins by cisplatin in human malignant glioblastoma A172 and T98G cells. To investigate whether the expression of p53 and the p53-response gene, p21/WAF1/CIP1, would affect the cytotoxicity of cisplatin, we performed Western blot analysis and found that the accumulation of p53 and p21 proteins was increased in A172 cells in a time-dependent manner but not in T98G cells (Fig. 2). These data indicate that cisplatin induced the accumulation of p53, followed by p21/WAF1/CIP1, in A172 cells harboring a functional *p53* gene; however, this was not the case in T98G cells harboring a mutant *p53* gene.

Expression of p53 and p21/WAF1/CIP1 proteins and apoptotic cell death in p53-infected T98G cells. To study whether the different sensitivity of A172 and T98G cells to cisplatin was associated with mutation of the p53 gene, including the signal pathways leading to apoptosis, T98G cells were transfected with adenoviral vector containing wild-type p53 (Ad.p53). Western blot analysis showed that introduction of Ad.p53 in T98G cells increased the expression of the p53 protein, maximally at 24 h (Fig. 3A), and treatment of cisplatin dramatically decreased the viability of Ad.p53 transfected T98G cells (Fig. 3B). To examine the effect of cisplatin on the apoptotic cell death of p53-transfected T98G cells, we carried out flow cytometry analysis of A172, T98G and Ad.p53-infected T98G cells after 48 h of cisplatin exposure by measuring hypodiploid DNA content stained with PI and

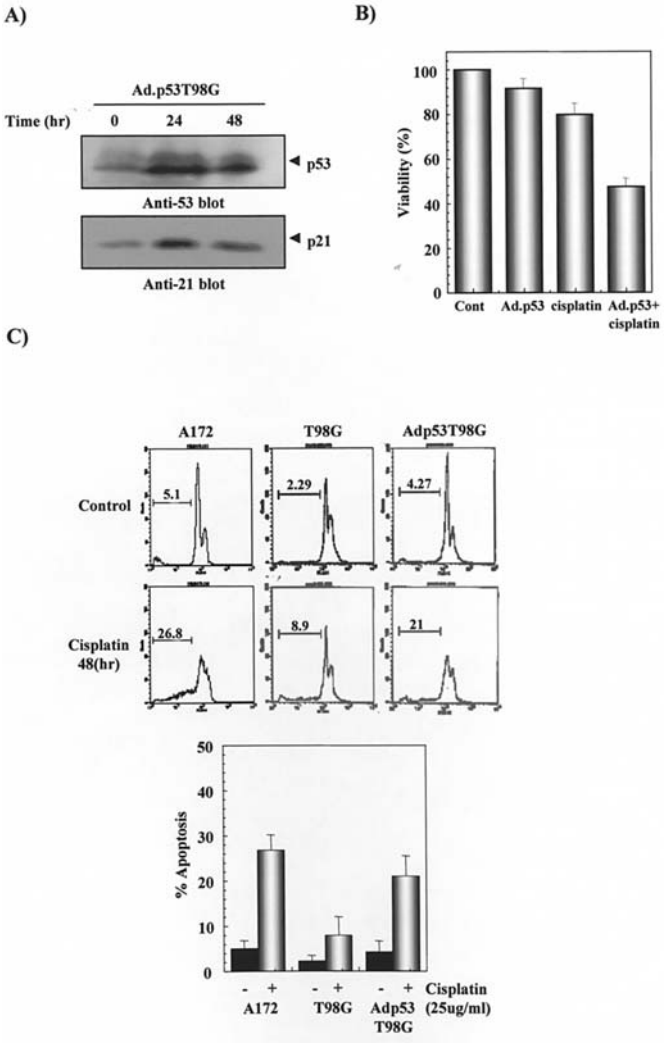

Figure 3. Introduction of wild-type p53 induced cisplatin chemosensitivity in T98G cells. (A) Cisplatin modulates the expression of p53 and p21 proteins in p53-infected T98G cells. Cells were treated with cisplatin (25 μg/ml) for different time periods and lysate from the cells was used to measure the p53 and p21 proteins by Western blotting using anti-p53 and p21 antibodies. (B) Ad.p53-transduced T98G cells showed cytotoxicity to cisplatin (25 μg/ml). Cell viability was determined by MTT assay after treatment of the cells with cisplatin (25 μg/ml) for 48 h. (C) Transduction of Ad.p53 increased the apoptosis in T98G cells. Cells were treated for 48 h with or without cisplatin (25 μg/ml). The number of apoptotic cells was measured by flow cytometry as described in Materials and methods. The region to the left of the G0/G1 peak was defined as cells undergoing apoptosis-associated DNA degradation. In bar graphs, the data represent mean ± SD for three independent experiments.

found that cisplatin markedly enhanced the proportion of apoptotic cells in A172 and Ad.p53 T98G cells, but not in T98G cells (Fig. 3C). Therefore, these data indicate that induction of p53 expression is critical for chemosensitivity of cisplatin in these cells.

Activation of caspase-3 and PARP degradation in cisplatin-treated A172, T98G cells and Ad.p53-infected T98G cells. To investigate whether the expression of p53 in A172 and T98G cells affected cisplatin-induced apoptotic signaling pathway, the activation of caspase-3 and PARP cleavage was measured by Western blot analysis. As shown in Fig. 4A, cisplatin significantly activated caspase-3 in a time-dependent manner

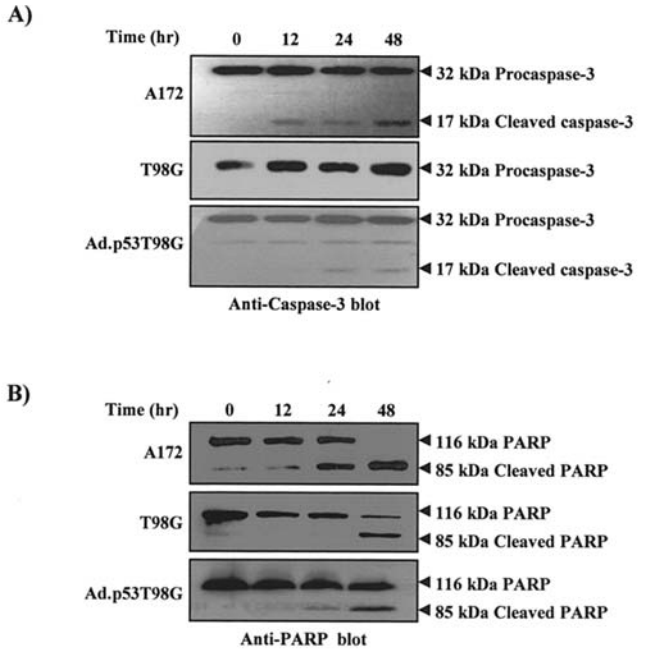

Figure 4. Cisplatin activates caspase-3 (A) and PARP cleavage (B) in A172 and p53-infected T98G cells. Cells were treated with 25 μg/ml cisplatin for different time periods and equal amounts of protein from cell lysate were separated by 15% (A) and 12.5% (B) SDS-PAGE and analyzed by immunoblotting with anti-caspase-3 (A) and anti-PARP antibodies (B).

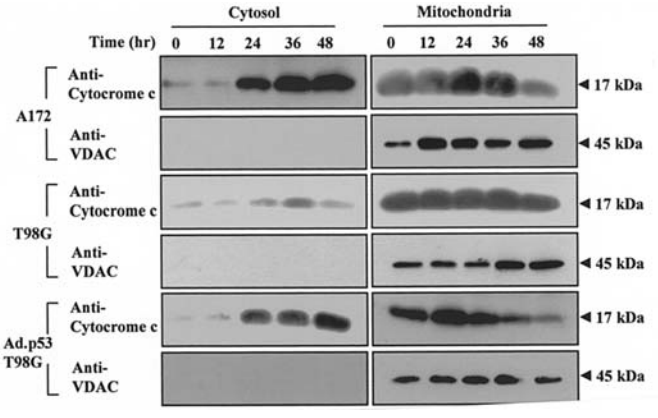

Figure 5. Cisplatin induces cytochrome c release from mitochondria in A172 and p53 infected T98G cells. Cytosolic or mitochondrial fraction was used for Western blot analysis with anti-cytochrome c or VDAC, a specific protein of mitochondria. Cells were treated with cisplatin (25 μg/ml) for different time periods and equal amounts of protein from cell lysates were resolved by 15% SDS-PAGE. The immuno-reactive signals were visualized by ECL kit (Amersham).

in A172 and Ad.p53-infected T98G cells but not in T98G cells. We next examined whether PARP, an intracellular biosubstrate of caspase-3 protease, was cleaved after cisplatin treatment and found that cleavage of 119 kDa PARP to 85 kDa was detected at 24 h after cisplatin treatment and maintained for 48 h in A172 and p53-infected T98G cells (Fig. 4B). On the other hand, cleavage of PARP in T98G cells was detected at 48 h after cisplatin treatment (Fig. 4B). These data suggest that cisplatin-induced apoptosis was accomplished through p53-mediated caspase-3 activation.

p53-mediated cytochrome c release in cisplatin-treated A172, T98G cells and p53-infected T98G cells. To elucidate the role of mitochondria in cisplatin-induced caspase activation by expression of the p53 protein, we examined mitochondrial cytochrome c release in A172, T98G and Ad.p53-infected T98G cells: the release of cytochrome c into cytosol from mitochondria was examined by Western blot analysis in cisplatin-treated cells. As shown in Fig. 5, cytosolic cytochrome c was detected at 12 h and further increased in a time-dependent manner for 48 h, whereas mitochondrial cytochrome c was concurrently dramatically decreased, verified in mitochondrial fraction with anti-VADC antibody in A172 and p53-infected T98G cells. In contrast, cytochrome c release of T98G cells after cisplatin treatment was only slightly increased, in comparison with p53-infected T98G cells (Fig. 5). These data suggest that cytochrome c release due to mitochondrial dysfunction may be responsible for the induction of caspase-3-dependent apoptosis of functional p53-harboring glioma cells by cisplatin.

Discussion

Although the action mechanism of cisplatin in various cancer cells is relatively well understood and known to result in the induction of apotosis, its effect on brain tumor cells remains poorly characterized. Accordingly, we attempted to identify and functionally characterize the p53 status that may be involved in cisplatin-induced cell death (39-41). In order to more clearly understand the role of p53 in cisplatin-induced apoptosis, we employed two glioma cell lines, A172 and T98G which have wild-type and mutant-type p53 respectively, and examined the possible association of defect in the signal pathways with difference of sensitivity to cisplatin treatment. Indeed, the results clearly showed that cisplatin induced p53-mediated apoptosis in A172 through the activation of caspase-3-like protease; however, it only had a marginal effect on T98G human glioblastoma cells. The introduction of Ad.p53 into T98G cells increased the apoptosis to cisplatin, thus revealing that cisplatin-induced chemosensitivity was at least partly mediated by the induction of p53 in glioma cells.

It is well known that p53 has to be accumulated and activated in response to various DNA-damaging agents in order to induce apoptotic cell death (42) and that p21 is a universal and potent cdk inhibitor under the control of p53 product (43). Our results clearly showed that cisplatin increased the p53 level in A172 cells followed by the induction of p21 expression, whereas cisplatin failed to accumulate and activate p53 and p21 proteins in T98G cells. To identify the involvement of p53 in the difference of chemosensitivity between A172 and T98G cells, we transfected wild-type p53, Ad.p53, in T98G cells and measured the induction of p53 and p21 and the proportion of apoptotic cell death. Our results clearly revealed that tranduction of Ad.p53 enhanced the expression of p53 and p21, as well as cisplatin cytotoxity in T98G cells, thus strongly suggesting that cisplatin induced p53-mediated apoptosis of both human malignant glioblastoma A172 and T98G cells via accumulation and activation of p53.

To clearly understand the role of p53 in cisplatin-induced apoptosis, we assessed caspase-3 activation, PARP cleavage and mitochondrial cytochrome c release after cisplatin

treatment and found that cisplatin induced conversion of procaspase-3 to active caspase-3 protease, PARP cleavage and mitochondrial cytochrome c release in A172 and Ad.p53-transfected T98G cells, whereas T98G cells failed to show cleavage of caspase-3 and a lesser extent of PARP cleavage and mitochondrial cytochrome c release. Therefore, accumulation and activation of the p53 protein appear to result in p53-dependent apoptotic cell death by activation of caspase-3 protease via the release of cytochrome c from mitochondria in A172 and Ad.p53-transfected T98G cells. These results suggest that there may be two mechanisms for understanding the cisplatin-induced apoptotic signaling pathway in human glioblastoma cell lines: one is that cisplatin induces p53-dependent apoptotic cell death and the other is a different pathway, leading to cleavage of PARP and the release of cytochrome c from the mitochondria.

In conclusion, we demonstrated that p53 played a critical role in cisplatin-induced apoptosis and accumulation and activation of p53 might trigger p53-dependent expression of p21, activation of caspase-3 protease, cleavage of PARP and cytochrome c release in A172 and Ad.p53-transfected T98G human glioblstoma cells. Therefore, the introduction of wild-type p53 in glioma cells lacking functional p53 might be an effective means of extending cisplatin's therapeutic significance.

References

1. Heesters MA, Koudstaal J, Go KG and Molenaar WM: Analysis of proliferation and apoptosis in brain gliomas: prognostic and clinical value. J Neurooncol 44: 255-266, 1999.
2. Vick NA, Khandekar JD and Bigner DD: Chemotherapy of brain tumors. Arch Neurol 34: 523-526, 1997.
3. Katayama K, Wada K, Nakajima A, Karmisaki Y and Mayumio T: Nuclear receptors as targets for drug development. J Pharmacol Sci 97: 171-176, 2005.
4. Prados MD and Levin V: Biology and treatment of malignant glioma. Semin Oncol 27 (suppl 6): 1-10, 2000.
5. Kondo Y, Hollingsworth EF and Kondo S: Molecular targeting for malignant gliomas. Int J Oncol 24: 1101-1109, 2004.
6. Madajewicz S and Chowhan N: Therapy for patients with high grade astrocytoma using intraarterial chemotherapy and radiation therapy. Cancer 88: 2350-2356, 2000.
7. Efferth T and Grassmann R: Impact of viral oncogenesis on responses to anti-cancer drugs and irradiation. Crit Rev Oncog 11: 165-187, 2000.
8. Yoshida T and Kawano N: Clinical cure of glioblastoma-two case reports. Neurol Med Chir 40: 224-229, 2000.
9. Maltzman W and Cxyxyk L: UV irradiation stimulates levels of p53 cellular tumor antigen in non-transformed mouse cells. Mol Cell Biol 4: 1689-1694, 1984.
10. Lowe SW, Ruley HE, Jacks T and Housman DE: p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 74: 957-967, 1993.
11. Lowe SW, Schmitt EM, Smith SW, Osborne BA and Jacks T: p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature 362: 847-849, 1993.
12. Kastan MB, Zhan Q, El-Deiry WW, Carrier F, Jacks T, Walsh WV, Plunkett BS, Vogelstein B and Fornace AJ: A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71: 587-597, 1992.
13. Fritsche M, Haessler C and Brandner G: Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. Oncogene 8: 307-318, 1993.
14. Adimoolam S and Lin CX: The p53-regulated cyclin-dependent kinase inhibitor, p21 (cip1, waf1, sdi1), is not required for global genomic and transcription-coupled nucleotide excision repair of UV-induced DNA photoproducts. J Biol Chem 276: 25813-25822, 2001.
15. Miyashita T and Reed JC: Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 80: 293-299, 1995.
16. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, Tokino T, Taniguchi T and Tanaka N: Noxa, a BH3- only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science 288: 1053-1058, 2000.
17. Oda K, Aradawa H, Tanaka T, Matsuda K, Tanikawa C, Mori T, Nishimori H, Tamai K, Tokino T, Nakamura Y and Taya Y: p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. Cell 102: 849-862, 2000.
18. Green DR and Reed JC: Mitochondria and apoptosis. Science 281: 1309-1312, 1998.
19. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES and Wang X: Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91: 479-489, 1997.
20. Saleh A, Srinivasula SM, Acharya S, Fishel R and Alnemri ES: Cytochrome c and dATP-mediated oligomerization of Apaf-1 is a prerequisite for procaspase-9 activation. J Biol Chem 274: 17941-17945, 1999.
21. Hu Y, Benedict MA, Ding L and Nunez G: Role of cytochrome c and dATP/ATP hydrolysis in Apaf-1-mediated caspase-9 activation and apoptosis. EMBO J 18: 3586-3595, 1999.
22. Yu Y and Little JB: p53 is involved in but not required for ionizing radiation-induced caspase-3 activation and apoptosis in human lymphoblast cell lines. Cancer Res 5: 4277-4281, 1998.
23. Ding HF, McGill G, Rowan S, Schmaltz C, Shimamura A and Fisher DE: Oncogene-dependent regulation of caspase activation by p53 protein in a cell-free system. J Biol Chem 273: 28378-28383, 1998.
24. Fuchs EJ, McKenna KA and Bedi A: p53-dependent DNA damage-induced apoptosis requires Fas/APO-1-independent activation of CPP32beta. Cancer Res 57: 2550-2554, 1997.
25. Henkels KM and Turchi JJ: Cisplatin-induced apoptosis proceeds by caspase-3-dependent and -independent pathways in cisplatin-resistant and -sensitive human ovarian cancer cell lines. Cancer Res 59: 3077-3083, 1999.
26. Soengas MS, Alarcon RM, Yoshida H, Giaccia AJ, Hakem R, Mak TW and Lowe SW: Apaf-1 and caspase-9 in p53-dependent apoptosis and tumor inhibition. Science 284: 156-159, 1999.
27. Biroccio A, Bufalo DD, Ricca A, D'Angelo C, D'Orazi G, Sacchi A, Soddu S and Zupi G: Increase of BCNU sensitivity by wt-p53 gene therapy in glioblastoma lines depends on the administration schedule. Gene Ther 6: 1064-1072, 1999.
28. Kano H, Arakawa Y, Hashimoto N, Takahashi JA, Nozaki K, Kawabata Y, Takatsuka K, Kageyama R and Ueba T: Over-expression of RFT induces G1-S arrest and apoptosis via p53/p21(waf1) pathway in glioma cell. Biochem Biophys Res Commun 317: 902-908, 2004.
29. Tanaka H, Arakawa H, Yamaguchi T, Shiraish K, Fukuda S, Matsui K, Takei Y and Nakamura Y: A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. Nature 404: 42-49, 2000.
30. El-Deiry WS, Harper JW, O'Connor PM, Velculescu VE, Canman CE, Jackman J, Pietenpol JA, Burrell M, Hill DE, Wang Y, Wiman KG, Mercer WE, Kastan MB, Kohn KW, Elledge SJ, Kinzler KW and Vogelstein B: WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. Cancer Res 54: 1169-1174, 1994.
31. Gross MW, Kraus A, Nashwan K, Mennel HD, Engenhart-Cabillic R and Schlegel J: Expression of p53 and p21 in primary glioblastomas. Strahlenther Onkol 181: 164-171, 2005.
32. Mazzatti DJ, Lee YJ, Helt CE, O'Reilly MA and Keng PC: p53 modulates radiation sensitivity independent of p21 transcriptional activation. Am J Clin Oncol 28: 43-50, 2005.
33. Pawlik TM and Keyomarsi K: Role of cell cycle in mediating sensitivity to radiotherapy. Int J Radiat Oncol Biol Phys 59: 928-942, 2004.
34. Kartalou M and Essigmann JM: Mechanisms of resistance to cisplatin. Mutat Res 478: 23-24, 2001.
35. Chu G: Cellular responses to cisplatin. The roles of DNA-binding proteins and DNA repair. J Biol Chem 269: 787-790, 1994.
36. Ferreira CG, Span SW, Peters GJ, Kruyt FA and Giaccone G: Chemotherapy triggers apoptosis in a caspase-8-dependent and mitochondira-controlled manner in the non-small cell lung cancer cell line NCI-H460. Cancer Res 60: 7133-7141, 2000.

37. Zeimet AG, Riha K, Berger J, Widschwendter M, Hermann M and Daxenbichler G: New insights into p53 regulation and gene therapy for cancer. Biochem Pharmacol 60: 1153-1163, 2001.
38. Gjerset RA, Turla ST, Sobol RE, Scalise JJ, Mercola D, Collins H and Hopkins PJ: Use of wild-type p53 to achieve complete treatment sensitization of tumor cells expressing endogenous mutant p53. Mol Carcinog 14: 275-285, 1995.
39. Horowitz J: Adenovirus-mediated p53 gene therapy: overview of preclinical studies and potential clinical applications. Curr Opin Mol Ther 1: 500-509, 1999.
40. Schmidt F, Groscurth P, Kermer M, Dichgans J and Weller M: Lovastatin and phenylacetate induce apoptosis, but not differentiation, in human malignant glioma cells. Acta Neuropathol 101: 217-224, 2001.
41. Reddy RM, Tsai WS, Ziauddin MF, Zuo J, Cole GW Jr, Maxhimer JB, Fang B, Schrump DS and Nguyen DM: Cisplatin enhances apoptosis induced by a tumor-selective adenovirus expressing tumor necrosis factor-related apoptosis-inducing ligand. J Thorac Cardiovasc Surg 128: 883-891, 2004.
42. Datta K, Shah P, Srivastava T, Mathur SG, Chattopadhyay P and Sinha S: Sensitizing glioma cells to cisplatin by abrogating the p53 response with antisense oligonucleotides. Cancer Gene Ther 11: 525-531, 2004.
43. Lincet H, Poulain L, Remy JS, Deslandes E, Duigou F, Ganduchon P and Staedel C: The p21(cip1/waf1) cyclin-dependent kinase inhibitor enhances the cytotoxic effect of cisplatin in human ovarian carcinoma cells. Cancer Lett 161: 17-26, 2000.