Cancer Chemother Pharmacol (2002) 49: 438–444
DOI 10.1007/s00280-002-0452-4

ORIGINAL ARTICLE

Iwao Iwasaki · Hisahiko Sugiyama
Shinsaku Kanazawa · Hiromichi Hemmi

Establishment of cisplatin-resistant variants of human neuroblastoma cell lines, TGW and GOTO, and their drug cross-resistance profiles

Received: 6 July 2001 / Accepted: 13 February 2002 / Published online: 4 April 2002
© Springer-Verlag 2002

Abstract *Purpose:* The emergence of multidrug resistance (MDR) in neuroblastoma is a critical issue for chemotherapy. In order to study low-level MDR, we developed variants derived from two neuroblastoma cell lines, TGW and GOTO, by exposure to low doses of cisplatin (CDDP). The cross-resistance to other cytotoxic agents and expression of MDR-related proteins in the variants and their clones were examined. *Materials and methods:* Cells were exposed to 3 or 10 μ*M* CDDP and three variants were obtained from each cell line, TGW and GOTO. Clones TR1 and TR2, derived from the TGW variants, were also established. Cytotoxicity was determined using a dye-staining method. Expression of MDR-related proteins was detected by immunoblotting. *Results:* Resistant variants exhibited 1.1- to 2.5-fold increased resistance to CDDP, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), doxorubicin and vincristine. The cytotoxicity of these agents varied between clones of resistant variants. The microsatellite profiles of the TR1 clones differed, indicating that the TR1 variant comprised a heterogeneous cell population. The cytotoxicities of cytosine β-D-arabinofuranoside (Ara-C) and chlorambucil in these variants and clones were similar to those in the parent cells. No significant changes in the cellular levels of MDR1, MRP, hMLH1 and hMSH2 were detected in the TGW variants. Cyclosporin A increased the sensitivity of both parental cell lines and the variants to doxorubicin and vincristine, but not to CDDP or MNNG. *Conclusions:* Ara-C and chlorambucil may be useful for the treatment of neuroblastoma exhibiting an MDR phenotype. These CDDP-resistant variants and clones may be useful for studying the mechanisms of low-level drug resistance in neuroblastoma.

Keywords Neuroblastoma cell line · Multidrug resistance · Cisplatin · Circumvention

I. Iwasaki · H. Sugiyama · S. Kanazawa · H. Hemmi (✉)
Department of Molecular Biology,
Toho University School of Medicine,
5-21-16 Ohmori-Nishi, Ohta-Ku,
Tokyo 143-8540, Japan
E-mail: hhemmi@med.toho-u.ac.jp
Tel.: +81-3-37624151
Fax: +1-3-37646923

Introduction

Neuroblastoma, a malignant tumor of neural crest origin, is the most common extracranial solid neoplasm of childhood [36]. The prognosis for patients with N-*myc*-amplified neuroblastoma varies with the effectiveness of chemotherapy and the 5-year survival rate has improved to 43.9% [34]. A favorable prognosis has also been reported in other advanced neuroblastomas with several therapeutic regimens [8].

Chemotherapeutic agents such as cisplatin (CDDP), THP-Adriamycin, vincristine, and melphalan are effective in childhood neuroblastomas. However, a recently emerging problem is that of multidrug resistance (MDR) in residual tumor cells after chemotherapy and/or surgical resection. The mechanism of MDR in neuroblastoma is thought to be associated with drug-pumping proteins such as P-glycoprotein encoded by the *MDR1* gene [7, 17], multidrug resistance-associated protein (MRP) [4, 6], and canalicular multispecific organic anion transporter (cMOAT) [29]. Recently, the breast cancer resistant protein (BCRP) and the ATP-binding cassette gene have been reported to be associated with MDR in adult breast and ovarian cancer cells [3, 10, 28]. In addition to these ATP-binding proteins, low level MDR to DNA-damaging agents including CDDP, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), and doxorubicin, has been reported in cancer cells deficient in DNA mismatch repair (MMR), including those of colon and ovarian origin [1, 2, 11, 32, 38]. Low-level MDR has

been demonstrated to be sufficient for resistant tumor regeneration in a mouse model [14]. Furthermore, after treatment of ovarian cancer with CDDP, many relapsed tumors are MMR deficient, indicating that CDDP results in selective enrichment of resistant tumor cells [40].

Altered drug sensitivity in colon and ovarian cancer cells has been identified using paired cell lines with similar genetic and cytogenetic compositions [11, 26]. However, few such neuroblastoma cell lines are available [15, 37, 42]. A series of human neuroblastoma cell lines exhibiting a low-level MDR phenotype would be desirable for studying the mechanisms of MDR. In the present study, to establish such human neuroblastoma cell lines with an MDR phenotype, six CDDP-resistant variants of the two human neuroblastoma cell lines, GOTO [32] and TGW [35], were established by repeated treatment with different doses of CDDP, and clones from two resistant variants were also isolated. The sensitivities of these variants and clones to cytotoxic agents and the mechanism of the MDR phenotype were examined. We demonstrated that altered sensitivity to commonly used chemotherapeutic agents for neuroblastoma was induced by CDDP, but that no significant change in sensitivity occurred in response to DNA polymerase inhibitors. Our results indicate that these variants and their clones may be useful for studying mechanisms of low-level MDR.

Materials and methods

Anticancer agents

Chlorambucil, CDDP, cyclosporin A (CsA), cytosine β-D-arabinofuranoside (Ara-C), doxorubicin, MNNG and vincristine were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Cells and cell culture

The human neuroblastoma cell lines, TGW and GOTO, obtained from the Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan), were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum as described previously [18]. Cells were removed from the surface of culture vessels by trypsinization, washed with phosphate-buffered saline (PBS) consisting of (in millimoles) NaCl 136.9, KCl 2.7, Na_2PO_4 8.1, and KH_2PO_4 1.5 (pH 7.3), and resuspended in 5 ml culture medium. Aliquots (0.5 ml) of the cell suspension were added to 4.5 ml fresh medium in 25-cm^2 flasks. Cell passaging was performed once weekly.

Resistant variants

CDDP-resistant variants of TGW were obtained by exposure to CDDP as follows. TGW cultured at an initial density of 10^4 cells per 25-cm^2 flask containing 5 ml medium for 2 or 3 days, was treated with 3 or 10 μM CDDP for 1 week. Cells were then cultured in CDDP-free medium for 3 weeks to recover cell density. The above treatment was repeated three times. The variant that survived exposure to 10 μM CDDP was designated as TR1 and that which survived exposure to 3 μM as TR2. TR3 was obtained by a procedure identical to that for TR1 except a second CDDP treatment at 20 μM was applied. CDDP-resistant GOTO variants were obtained by a protocol similar to that for the TGW variants. For GR1, three exposures to 3 μM were used. For GR2 and GR3, four exposures to 3 and 10 μM, respectively, were used. These variants, which were cultured for more than 6 months from establishment, exhibited stable characteristics with respect to growth rate, morphology, drug resistance and expression of the two MMR proteins, hMSH2 and hMLH1.

Cell cloning

Single-cell suspensions (100 µl) of TR1 and TR2, at a density of ten cells per milliliter, were inoculated into wells of a 96-well culture plate. Culture was continued for 2 to 3 weeks until the cells were semiconfluent and the cells were then cultured further in larger culture vessels. After the cytotoxic assay, some of the clones were lost. To date, 26 of the original 38 TR1 clones and 30 of the 39 TR2 clones have survived.

Cytotoxic assay

Cytotoxicity of anticancer agents was determined using a dye-staining method as described previously [33] with minor modifications. Briefly, 10^4 cells were cultured in triplicate wells of a 96-well microplate for 24 h, treated with various concentrations of an agent for 1 h, washed twice with medium, and cultured for a further 6 days. After the cells had been fixed and stained with a crystal violet solution, absorbance at 610 nm was measured with a microplate reader (model 550, Bio-Rad Laboratories, Hercules, Calif.).

Immunoblotting

Exponentially growing cells were harvested and cell lysates were prepared as described previously [18] and stored at –80°C until use. Heat-denatured cell lysates were electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel. The gel was electroblotted to a polyvinylidene difluoride (PVDF) membrane (Hybond-P, Amersham Pharmacia Biotech). To visualize specific bands, ECL-plus (Amersham Pharmacia Biotech) with mouse monoclonal antibodies against MDR1 (Cappel, Biochemical Division, ICN Pharmaceuticals, Aurora, Ohio) and MRP (Chemicon International, Temecula, Calif.) were used as recommended by the suppliers. hMLH1 and hMSH2 proteins were detected with the affinity-purified IgG fraction of mouse monoclonal antibodies against hMSH2 and hMLH1 as described previously [25]. Anti-β-actin mouse monoclonal antibody (Sigma) was used as a loading control.

Detection of genome stability at a microsatellite locus

To determine genome stability after CDDP treatment, DNA was isolated with a DNAzol reagent (Life Technologies, Gaithersburg, Md.). DNA fragments amplified by polymerase chain reaction (PCR) with two locus markers, *Mfd26* and *Mfd47*, were subjected to microsatellite locus profile analysis using an automated DNA sequencer (Model 373S, Applera Company, Applied Biosystems, Foster City, Calif.) as described previously [21].

Statistical analysis

Student's *t*-test was performed on a computer using software (DA Stats) written by O. Nagata of Tokyo University, and the *P* values were calculated.

Results

To determine the degree of alteration in the sensitivity of the resistant variants induced by CDDP treatment, cells were treated with various concentrations of CDDP

for 1 h and cytotoxicity on day 7 determined with a dye-staining method. Dose-dependent cytotoxicity was observed in all cells examined. The 50% growth inhibitory concentration (IC_{50}) of CDDP was determined from at least three independent experiments (Table 1). The IC_{50} values in TR1, TR2 and TR3 derived from TGW were 2.1-, 1.6- and 1.9-fold higher, respectively, than that of the parent cells, and the values in GR1, GR2 and GR3 derived from GOTO, were 1.5-, 1.7-, and 1.1-fold higher, respectively. The *P* values of these, except a set of GOTO and GR3, were less than 0.01. The IC_{50} values of other cytotoxic agents including doxorubicin, MNNG, vincristine, Ara-C and chlorambucil were also determined. The variants showed a low level of resistance to doxorubicin, MNNG and vincristine, in addition to CDDP (Table 1). However, the IC_{50} values of Ara-C and chlorambucil in the variants examined were very similar to those in the respective parent cell lines. It is possible that the variants comprised heterogeneous populations, as the cells had selectively survived repeated CDDP treatment, and/or that CDDP-induced pleiotropic changes had occurred in the surviving cells.

The variants TR1 and TR2 were cloned without treatment, and 38 clones of TR1 and 39 clones of TR2 were obtained. The sensitivity of these clones to CDDP and MNNG was 0.3- to 4.4-fold that of the parental variant, respectively. The relative cytotoxicities in some clones are shown in Fig. 1. The IC_{50} values of CDDP, MNNG, vincristine and doxorubicin varied widely from 0.6- to 4-fold, while those of Ara-C and chlorambucil were in the narrow range of 0.4- to 1.2-fold. These results indicate that the variants comprised heterogeneous populations with respect to sensitivity to common therapeutic agents for neuroblastoma, including CDDP, vincristine and doxorubicin. Furthermore, Ara-C and chlorambucil may be effective against resistant tumor cells with an MDR phenotype.

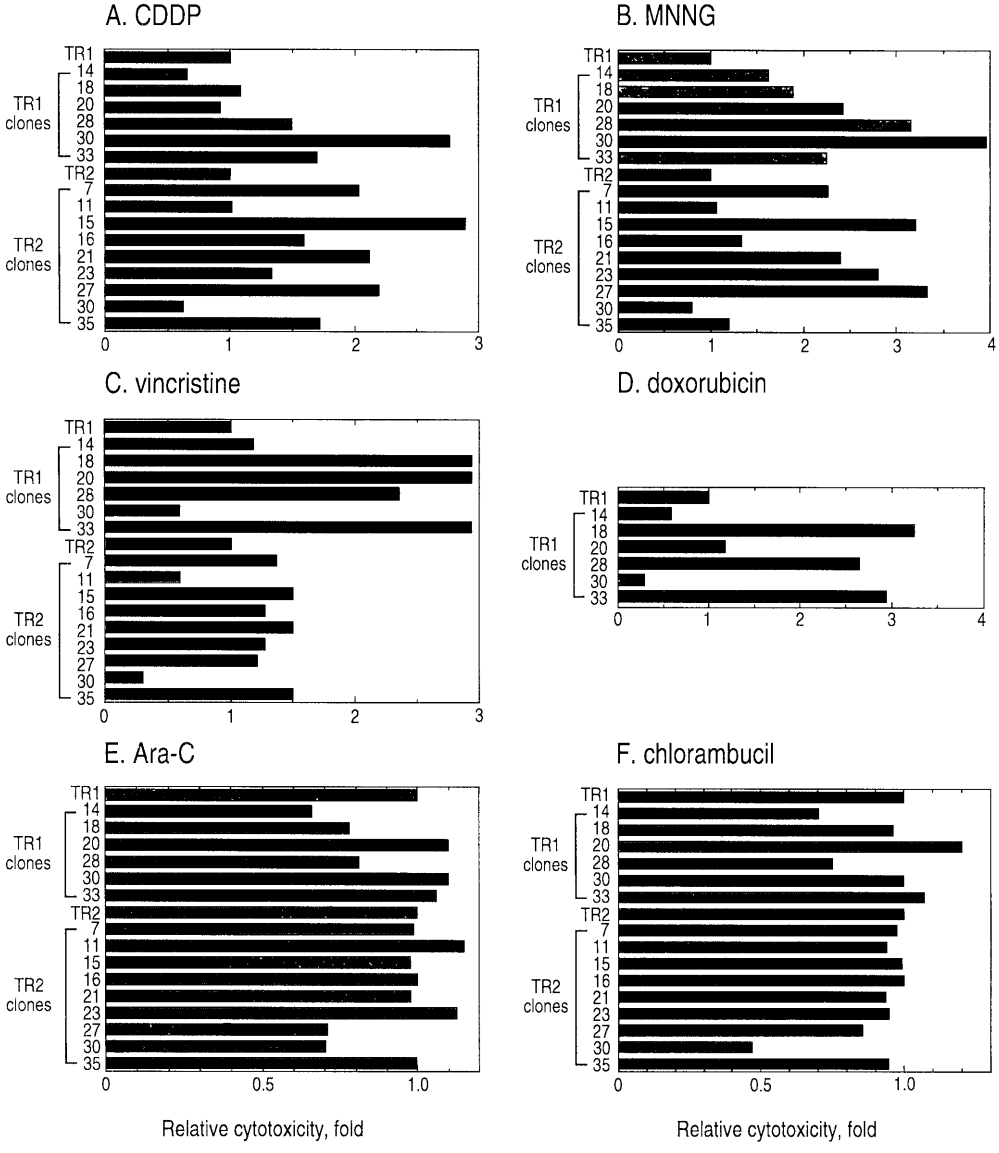

Fig. 1A–F. Comparison of the sensitivities of representative clones and their parental variants, TR1 and TR2, to cytotoxic agents. The IC_{50} values of CDDP (**A**), MNNG (**B**), vincristine (**C**), doxorubicin (**D**), Ara-C (**E**) and chlorambucil (**F**) in TR1, TR2, and their clones were determined using a dose-response curve. The relative cytotoxicity in a clone was derived from the IC_{50} value in relation to that in the parental variant, TR1 or TR2, and is expressed as fold increase. The relative cytotoxicity in TR1 or TR2 was defined as 1

Since the CDDP-resistant variants exhibited an MDR phenotype, we then examined the expression of the MDR-related proteins MDR1, MRP, hMSH2 and hMLH1. Immunoblot analysis showed no significant differences in the expression of MDR1 and MRP proteins between parent cells and resistant variants treated with CDDP or doxorubicin (Fig. 2A). Furthermore, hMLH1 and hMSH2 proteins were detected in these variants (Fig. 2B). Expression of hMLH1 and hMSH2 was also determined in 26 TR1 clones and 30 TR2 clones. The MMR proteins were detected in all clones tested (data not shown). These results indicate that induction of MDR1 and MRP or deficiency of MMR proteins may not contribute to the MDR phenotype in TGW variants. It is possible, however, that undetectable changes in *MDR1* gene expression may have affected low-level drug sensitivity. To clarify this, we examined the effect of CsA on the cytotoxicities of CDDP, MNNG, doxorubicin and vincristine in the variants and six clones of TR1. Altered cytotoxicity was observed in all parent cells and variants with the exception of GR2 (Fig. 3).

Cytotoxicities of CDDP and MNNG increased in both parent cells and variants, but cytotoxicity in the variants was never greater than that in the parent cells. The TR1 clones tended to be similar to TR1 with respect to sensitivity to CDDP and MNNG, although there was variability between clones (Fig. 4). In contrast, the cytotoxicities of doxorubicin and vincristine in the variants and clones were enhanced in the presence of CsA, with the exception of GR2 and TR1-30 (Figs. 3 and 4).

Because CDDP is known as a potent mutagen [5], we next investigated the alteration in DNA sequence in TR1 and the three TR1-derived clones. We selected two microsatellite loci, *Mfd26* located at 18q12 and *Mfd47* on chromosome 6. The microsatellite profiles of both loci in TR1 differed from those in TGW, and the profiles of the clones also differed from each other (Fig. 5). The profiles of GR1 and GR2 were also different from those of GOTO (data not shown). These results indicate that repeated exposure to low-dose CDDP induced alterations in the DNA sequence throughout the entire genome.

Discussion

Intensive chemotherapy of neuroblastomas has improved survival even in high-risk groups. However, neuroblastomas can acquire sustained drug resistance during chemotherapy. The spectrum of drugs to which resistance can be acquired is known to include melphalan, carboplatin, etoposide, THP-Adriamycin and vincristine [23, 24, 34, 37]. We developed resistant variants of the human neuroblastoma cell lines TGW and GOTO by repeated exposure to CDDP, and then selected clones of the variants derived. These cells exhibited resistance to CDDP, MNNG, doxorubicin and vincristine, chemotherapeutic agents commonly used in the treatment of neuroblastomas. However, the sensitivity of CDDP-treated resistant cells to Ara-C and chlorambucil (both generally used for treatment of leukemia) was similar to that of their parent cells. Our examination of the sensitivity of these antileukemic agents in neuroblastoma was based on studies in colorectal cancer.

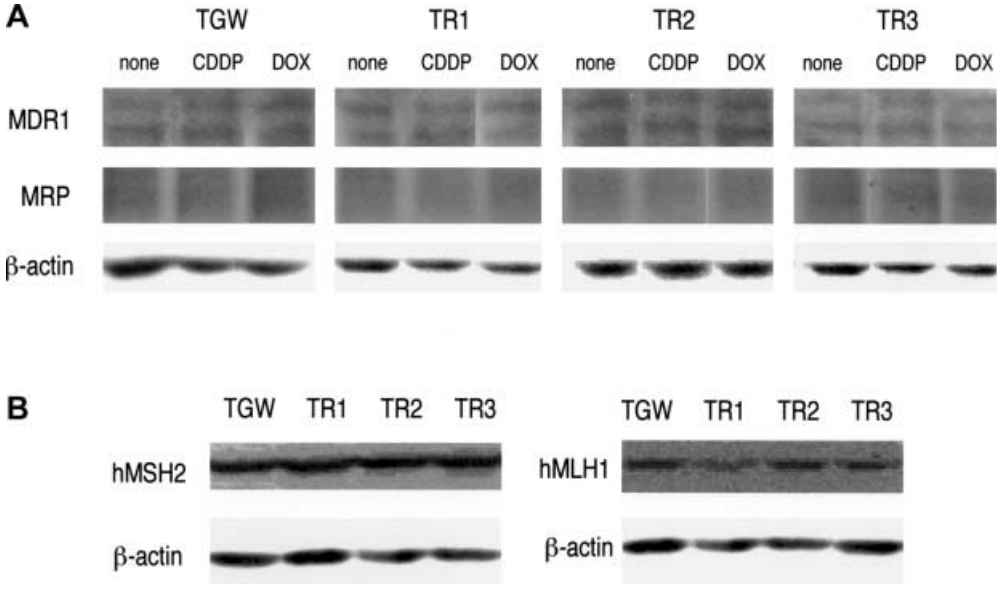

Fig. 2A, B. Immunoblotting does not indicate any significant alteration in MDR-related protein expression. **A** MDR1 and MRP protein expression. Cells were treated with 20 μ*M* CDDP or 20 μ*M* doxorubicin for 1 h, cultured for 72 h, and harvested. Cell lysates were electrophoresed in a 10% SDS-polyacrylamide gel and electroblotted to a PVDF membrane. The membrane was treated with anti-MDR1 antibody or anti-MRP antibody as primary antibody. The specific band was visualized with an ECL-plus kit and photographed. The membrane was reprobed and anti-*β*-actin antibody was used as a loading control. The reverse images are shown. **B** hMSH2 and hMLH1 proteins. Exponentially growing cells were harvested and subjected to immunoblot analysis. Anti-*β*-actin antibody was used as a loading control

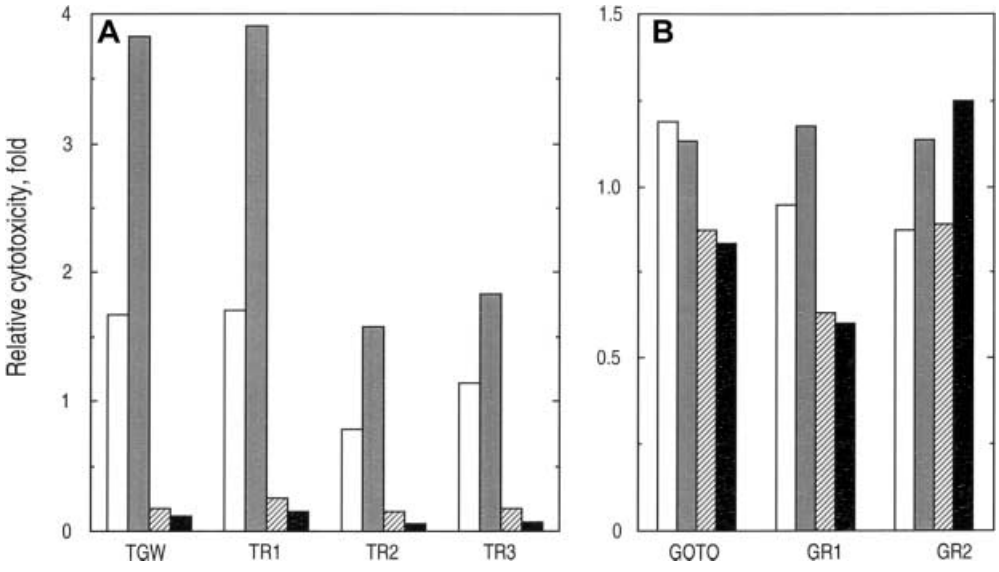

Fig. 3A, B. Effect of CsA on the cytotoxicity of CDDP, MNNG, doxorubicin and vincristine in TGW and GOTO, and their resistant variants. Cytotoxicities of CDDP (*white bars*), MNNG (*shaded bars*), doxorubicin (*hatched bars*), and vincristine (*black bars*) were determined in the absence or presence of 10 μ*M* CsA (CsA concentration had no effect on cell growth). Relative cytotoxicity was estimated from the following formula: IC_{50} value in the presence of CsA/ IC_{50} value in the absence of CsA

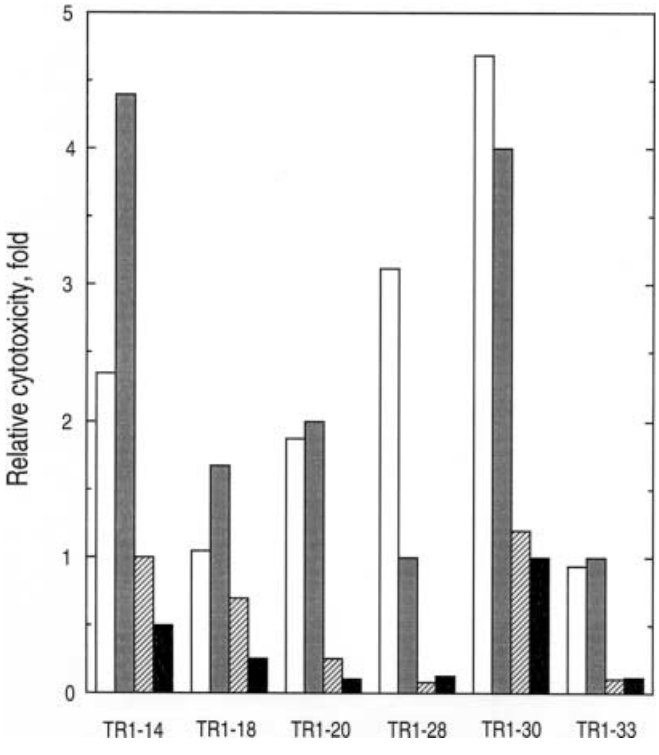

Fig. 4. Effect of CsA on cytotoxicity of CDDP, MNNG, doxorubicin and vincristine in TR1 clones. Cytotoxicities of CDDP (*white bars*), MNNG (*shaded bars*), doxorubicin (*hatched bars*), and vincristine (*black bars*) were determined in the absence or presence of 10 μ*M* CsA (CsA concentration had no effect on cell growth). Relative cytotoxicity was estimated from the following formula: IC_{50} value in the presence of CsA/IC_{50} value in the absence of CsA

MMR deficiency confers resistance to CDDP [1, 11], carboplatin [2], and alkylating agents including MNNG [26, 38], doxorubicin [11], etoposide [2] and vinca alkaloids [38]. The spectrum of drug resistance in colon and ovarian cancers is largely similar to that in neuroblastoma. We have demonstrated that Ara-C can circumvent MDR in an MMR-deficient colorectal cancer cell line [38]. Furthermore, hMLH1-deficient ovarian tumors frequently recur after CDDP treatment [40]. Thus, it is possible that exposure of TGW and GOTO cells to CDDP induces or results in enrichment of MMR-deficient cells. However, we could not detect hMLH1- or hMSH2-deficient cells in resistant variants or any clones examined. Thus, it is unlikely that CDDP exposure induces MMR deficiency in neuroblastoma cells.

Increased expression of MDR-related proteins such as MDR1, BCRP, and MRPs has been demonstrated in tumor cells with an MDR phenotype. MDR1 was detected in both parent and resistant cells in our study. No significant increase was observed in CDDP- and doxorubicin-treated cells. CsA, an inhibitor of MDR1 [16] and BCRP [9, 30], sensitized resistant variants and parent cells to doxorubicin and vincristine cytotoxicity. It is possible that BCRP, but not MDR1, participates in the MDR phenotype in neuroblastoma. MRP was not detected in these cells by immunoblotting. These results indicate that MDR1 and MRP do not contribute to acquisition of sustained resistance by the variants. Semaphorin E [41] and other members of the MRP family [19, 27, 29, 39] have been identified as MDR-related proteins that alter chemosensitivity to CDDP. Further, Bader et al. have demonstrated that, in neuroblastoma, the MDR phenotype is caused by multiple resistance factors [4]. Low-level MDR in neuroblastoma may be a result of multiple events involving MDR-related proteins induced by exposure to a cytotoxic agent.

More recently, dysfunctional p53 in recurrent neuroblastomas has been found to confer an MDR phenotype [24, 37]. Mutation in the *p53* gene in primary neuroblastomas is very rare. However, loss of p53 function or the *p53* gene is frequently observed in recurrent tumors with an MDR phenotype after chemotherapy. Keshelava et al. [24] have demonstrated that neuroblastoma cell lines with disrupted p53 function (by

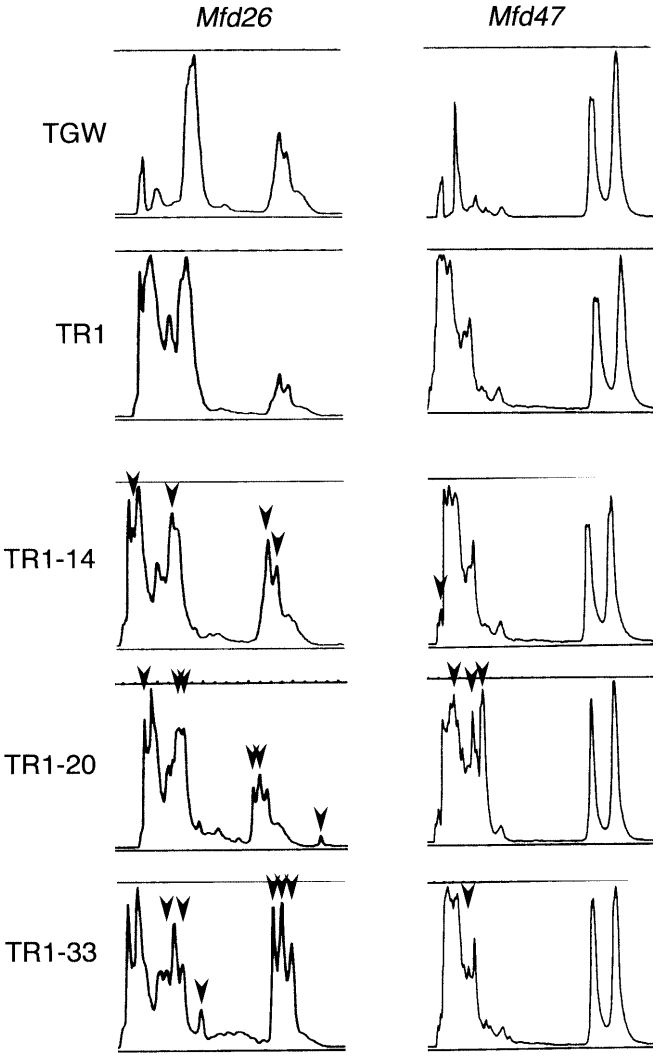

Fig. 5. Profiles of microsatellite loci in TGW, TR1 and TR1 clones. DNAs isolated from TGW, TR1, TR1-14, TR1-20 and TR1-33 were amplified by PCR with primers for the microsatellite loci *Mfd26* and *Mfd47*. The PCR products were analyzed using an automated DNA sequencer with Genotyper software (Applied BioSystems). *Arrowheads* indicate the presence of a major change in the clones

transfection of an HPV E6 expression vector) display resistance to melphalan, carboplatin and etoposide. TGW was established in culture from a xenograft, TNB1 [35], which was isolated from recurrent tumor tissue from a patient receiving chemotherapy and radiotherapy [22]. It is possible that TGW has a dysfunctional mutation in the *p53* gene. Preliminary studies of p53 function in TGW, TR1, TR2 and TR3 showed that p21 protein was inducible by doxorubicin treatment and was accompanied by increased nuclear p53. GOTO has been reported to have a wild-type p53 gene [20]. Further studies of p53 function and *p53* gene status in these resistant variants and clones are necessary.

Interestingly, sensitivities of TGW and its resistant variants, but not of GOTO, to CDDP and MNNG were decreased in the presence of a low dose (10 μM) of CsA. Since the nucleotides methylated by MNNG or platinum adducts generated by CDDP in DNA strands must be recognized by a heterodimer consisting of hMSH2 and hMSH6 [12, 13], CsA may interfere with the formation of these alkylated nucleotides or activation of the MMR signaling pathway, leading to cell cycle arrest and apoptosis. This type of interference may cause reduction in cytotoxicity. Another possibility is that CsA modulates p53 function in TGW cells. CsA stabilizes p53 through induction of heat-shock proteins [31]. However, the latter possibility is unlikely as induction of functional p53 should increase the cytotoxicity of these agents.

In conclusion, we developed six MDR variants and clones of TR1 and TR2 by repeated exposure to CDDP. The DNA sequence of microsatellite regions in these resistant cells differed from each other. The variants and clones exhibited different chemosensitivities to drugs commonly used for neuroblastoma therapy. These cell lines may be useful for studying mechanisms of MDR, other than involvement of ATP-binding proteins such as BCRP, MDR1, and MRPs, in neuroblastoma. Furthermore, the sensitivity to Ara-C and chlorambucil of all cells tested was relatively similar, indicating that these drugs may be useful for therapy of neuroblastoma, especially recurrent tumors.

Acknowledgements We thank Drs. Hiroyuki Shimatake and Keihachiro Hirano for their continuous support and Dr. Kimitoshi Kohno of the Department of Molecular Biology, University of Occupational and Environmental Health, School of Medicine, for useful suggestions. This work was supported in part by a Project Research Grant from Toho University School of Medicine (11-23).

References

1. Aebi S, Kurdi-Haidar B, Gordon R, Cenni B, Zheng H, Fink D, Christen RD, Boland CR, Koi M, Fishel R, Howell SB (1996) Loss of DNA mismatch repair in acquired resistance to cisplatin. Cancer Res 56:3087
2. Aebi S, Fink D, Gordon R, Kim HK, Zheng H, Fink JL, Howell SB (1997) Resistance to cytotoxic drugs in DNA mismatch repair-deficient cells. Clin Cancer Res 3:1763
3. Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M (1998) A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res 58:5337
4. Bader P, Schilling F, Schlaud M, Girgert R, Handgretinger R, Klingebiel T, Treuner J, Liu C, Niethammer D, Beck JF (1999) Expression analysis of multidrug resistance associated genes in neuroblastomas. Oncol Rep 6:1143
5. Beck DJ, Brubaker RR (1975) Mutagenic properties of cisplatinum (II) diammino-dichloride in *Escherichia coli*. Mutat Res 27:181
6. Bordow SB, Haber M, Madafiglio J, Cheung B, Marshall GM, Norris MD (1994) Expression of the multidrug resistance-associated protein (MRP) gene correlates with amplification and overexpression of the N-myc oncogene in childhood neuroblastoma. Cancer Res 54:5036
7. Bourhis J, Benard J, Hartmann O, Boccon-Gibod L, Lemerle J, Riou G (1989) Correlation of *MDR1* gene expression with chemotherapy in neuroblastoma. J Natl Cancer Inst 81:1401
8. Cohn SL, Look AT, Joshi VV, Holbrook T, Salwen H, Chagnovich D, Chesler L, Rowe ST, Valentine MB, Komuro H, Castleberry RP, Bowman LC, Rao PV, Seeger RC, Brodeur

GM (1995) Lack of correlation of N-*myc* gene amplification with prognosis in localized neuroblastoma: a Pediatric Oncology Group study. Cancer Res 55:721
9. Diah SK, Smitherman PK, Aldridge J, Volk EL, Schneider E, Townsend AJ, Morrow CS (2001) Resistance to mitoxantrone in multidrug-resistant MCF7 breast cancer cells: evaluation of mitoxantrone transport and the role of multidrug resistance protein family proteins. Cancer Res 61:5461
10. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, Ross DD (1998) A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci U S A 95:15665
11. Drummond JT, Anthoney A, Brown R, Modrich P (1996) Cisplatin and adriamycin resistance are associated with MutLα and mismatch repair deficiency in an ovarian tumor cell line. J Biol Chem 271:19645
12. Duckett DR, Drummond JT, Murchie AI, Reardon JT, Sancar A, Lilley DM, Modrich P (1996) Human MutSα recognizes damaged DNA base pairs containing O6-methylguanine, O4-methylthymine, or the cisplatin-d(GpG) adduct. Proc Natl Acad Sci U S A 93:6443
13. Fink D, Nebel S, Aebi S, Zheng H, Cenni B, Nehme A, Christen RD, Howell SB (1996) The role of DNA mismatch repair in platinum drug resistance. Cancer Res 56:4881
14. Fink D, Zheng H, Nebel S, Norris PS, Aebi S, Lin TP, Nehme A, Christen RD, Haas M, MacLeod CL, Howell SB (1997) In vitro and in vivo resistance to cisplatin in cells that have lost DNA mismatch repair. Cancer Res 57:1841
15. Fleming GF, Amato JM, Agresti M, Safa AR (1992) Megestrol acetate reverses multidrug resistance and interacts with P-glycoprotein. Cancer Chemother Pharmacol 29:445
16. Foxwell BM, Mackie A, Ling V, Ryffel B (1989) Identification of the multidrug resistance-related P-glycoprotein as a cyclosporin binding protein. Mol Pharmacol 36:543
17. Goldstein LJ, Galski H, Fojo A, Willingham M, Lai SL, Gazdar A, Pirker R, Green A, Crist W, Brodeur GM, Lieber M, Crossman J, Gottesman MM, Pastan I (1989) Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst 81:116
18. Hemmi H, Yamada K, Yoon U, Kato M, Taniguchi F, Tsuchida Y, Shimatake H (1995) Coexpression of the *myc* gene family members in human neuroblastoma cell lines. Biochem Mol Biol Int 36:1135
19. Hinoshita E, Uchiumi T, Taguchi K, Kinukawa N, Tsuneyoshi M, Maehara Y, Sugimachi K, Kuwano M (2000) Increased expression of an ATP-binding cassette superfamily transporter, multidrug resistance protein 2, in human colorectal carcinomas. Clin Cancer Res 6:2401
20. Jia LQ, Osada M, Ishioka C, Gamo M, Ikawa S, Suzuki T, Shimodaira H, Niitani T, Kudo T, Akiyama M, Kimura N, Matsuo M, Mizusawa H, Tanaka N, Koyama H, Namba M, Kanamaru R, Kuroki T (1997) Screening the p53 status of human cell lines using a yeast functional assay. Mol Carcinog 19:243
21. Kanazawa S (1999) A method for efficiently detecting the microsatellite instability phenotype in patients with sporadic colorectal cancer. J Med Soc Toho 46:15
22. Kaneko Y, Tsuchida Y, Maseki N, Takasaki N, Sakurai M, Saito S (1985) Chromosome findings in human neuroblastomas xenografted in nude mice. Jpn J Cancer Res 76:359
23. Keshelava N, Groshen S, Reynolds CP (2000) Cross-resistance of topoisomerase I and II inhibitors in neuroblastoma cell lines. Cancer Chemother Pharmacol 45:1
24. Keshelava N, Zuo JJ, Waidyaratne NS, Triche TJ, Reynolds CP (2000) p53 mutations and loss of p53 function confer multidrug resistance in neuroblastoma. Med Pediatr Oncol 35:563
25. Kikuchi Y, Takano S (1997) Preparation of antibodies against hMSH2 and hMLH1 and its cellular localization in human colon cancer cell lines. J Med Soc Toho 43:432
26. Koi M, Umar A, Chauhan DP, Cherian SP, Carethers JM, Kunkel TA, Boland CR (1994) Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine tolerance in colon tumor cells with homozygous *hMLH1* mutations. Cancer Res 54:4308
27. Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJ, Juijn JA, Baas F, Borst P (1997) Analysis of expression of *cMOAT* (*MRP2*), *MRP3*, *MRP4*, and *MRP5*, homologues of the multidrug resistance-associated protein gene (*MRP1*), in human cancer cell lines. Cancer Res 57:3537
28. Maliepaard M, van Gastelen MA, de Jong LA, Pluim D, van Waardenburg RC, Ruevekamp-Helmers MC, Floot BG, Schellens JH (1999) Overexpression of the BCRP/MXR/ABCP gene in a topotecan-selected ovarian tumor cell line. Cancer Res 59:4559
29. Matsunaga T, Shirasawa H, Hishiki T, Enomoto H, Kouchi K, Ohtsuka Y, Iwai J, Yoshida H, Tanabe M, Kobayashi S, Asano T, Etoh T, Nishi Y, Ohnuma N (1998) Expression of *MRP* and *cMOAT* in childhood neuroblastomas and malignant liver tumors and its relevance to clinical behavior. Jpn J Cancer Res 89:1276
30. Özvegy C, Litman T, Szakács G, Nagy Z, Bates S, Váradi A, Sarkadi B (2001) Functional characterization of the human multidrug transporter, ABCG2, expressed in insect cells. Biochem Biophys Res Commun 285:111
31. Paslaru L, Rallu M, Manuel M, Davidson S, Morange M (2000) Cyclosporin A induces an atypical heat shock response. Biochem Biophys Res Commun 269:464
32. Sekiguchi M, Oota T, Sakakibara K, Inui N, Fujii G (1979) Establishment and characterization of a human neuroblastoma cell line in tissue culture. Jpn J Exp Med 49:67
33. Shikano M, Inoue A, Nishide H, Ochiai J, Komatsu Y, Numao N, Hemmi H (1991) Growth inhibitory activity of ultimobranchial calcitonins in cultured mammalian cells. Tissue Cult Res Commun 10:31
34. Suita S, Zaizen Y, Kaneko M, Uchino J, Takeda T, Iwafuchi M, Utsumi J, Takahashi H, Yokoyama J, Nishihira H, Okada A, Kawa K, Nagahara N, Yano H, Tsuchida Y (1994) What is the benefit of aggressive chemotherapy for advanced neuroblastoma with N-*myc* amplification? A report from the Japanese Study Group for the Treatment of Advanced Neuroblastoma. J Pediatr Surg 29:746
35. Tsuchida Y, Sekiguchi S, Kaneko Y, Kanda N (1990) Origin of human neuroblastoma cell lines TGW and TNB1. FEBS Lett 263:191
36. Tsuchida Y, Hemmi H, Inoue A, Obana K, Taniguchi F, Hayashi Y, Kanda N, Shimatake H (1996) Genetic clinical markers of human neuroblastoma with special reference to N-*myc* oncogene: amplified or not amplified? Tumor Biol 17:65
37. Tweddle DA, Malcolm AJ, Bown N, Pearson AD, Lunec J (2001) Evidence for the development of p53 mutations after cytotoxic therapy in a neuroblastoma cell line. Cancer Res 61:8
38. Uchida I, Zhong X (1999) Differential cytotoxicity of anticancer agents in hMutSα-deficient and -proficient human colorectal cancer cells. Tissue Cult Res Commun 18:301
39. Uchiumi T, Hinoshita E, Haga S, Nakamura T, Tanaka T, Toh S, Furukawa M, Kawabe T, Wada M, Kagotani K, Okumura K, Kohno K, Akiyama S, Kuwano M (1998) Isolation of a novel human canalicular multispecific organic anion transporter, *cMOAT2*/*MRP3*, and its expression in cisplatin-resistant cancer cells with decreased ATP-dependent drug transport. Biochem Biophys Res Commun 252:103
40. Watanabe Y, Koi M, Hemmi H, Hoshiai H, Noda K (2001) A change in microsatellite instability caused by cisplatin-based chemotherapy of ovarian cancer. Br J Cancer 85:1064
41. Yamada T, Endo R, Gotoh M, Hirohashi S (1997) Identification of *semaphorin E* as a non-MDR drug resistance gene of human cancers. Proc Natl Acad Sci U S A 94:14713
42. Yasuno T, Matsumura T, Shikata T, Inazawa J, Sakabe T, Tsuchida S, Takahata T, Miyairi S, Naganuma A, Sawada T (1999) Establishment and characterization of a cisplatin-resistant human neuroblastoma cell line. Anticancer Res 19:4049