

Differential sensitivity to paclitaxel-induced apoptosis and growth suppression in paclitaxel-resistant cell lines established from HEC-1 human endometrial adenocarcinoma cells

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Abstract. To investigate acquired paclitaxel (PTX) resistance in cancer cells, we established five monoclonal PTX-resistant cell lines from HEC-1 human endometrial adenocarcinoma cells by means of long-term PTX-exposed cultures and limiting dilution cultures. The established PTX-resistant subclones showed apparent resistance to PTX-induced DNA fragmentation but not to PTX-induced growth suppression. None of the five PTX-resistant subclones showed apparent resistance to other anticancer drugs such as cisplatin, etoposide, 5-fluorouracil, pirarubicin-HCl, 4-hydroxy-cyclophosphamide or mitomycin C. Semiquantitative flow cytometric analysis revealed no apparent differential expression of 17 molecules that were previously reported to regulate apoptosis or drug resistance, between the five PTX-resistant subclones and the parent cells. Karyotyping analysis revealed common changes in chromosomes 4 and 18 in the five PTX-resistant subclones but not in the HEC-1 parent cells. These results indicate that PTX-induced growth suppression is regulated by different mechanisms from those involved in PTX-induced apoptosis. It was concluded that these established PTX-resistant subclones can be useful models in studies related to the prevention or treatment of recurrent cancers after PTX chemotherapy.

Introduction

Recent progress in the development of chemotherapy for advanced cancer has improved long-term overall survival rates. However, acquired drug-resistance during chemotherapy is now a significant clinical problem in advanced cancer patients because it inhibits complete remission. The prognosis of patients

with recurrent cancers or advanced malignant tumors would be remarkably improved, if the mechanisms involved in the acquisition of anticancer drug-resistance could be clarified and therapies to overcome drug-resistance or restore drug-sensitivity could be developed.

Paclitaxel (PTX) is an antineoplastic compound extracted from the Pacific yew tree, *Taxus brevifolia*. It binds to tubulin and inhibits the disassembly of microtubules, thereby resulting in the inhibition of cell division. PTX is now widely used to treat patients with various types of cancers such as lung, ovarian, breast, gastric, endometrial and cervical cancers. Combination chemotherapy with PTX and carboplatin is now the first line chemotherapy for ovarian and endometrial cancer patients. Unfortunately, acquired resistance to PTX in cancer patients has become a major clinical problem in cancer chemotherapy.

There have been many studies that have investigated the molecular mechanisms involved in PTX-resistance in cancer cells. Increased β -1 integrin expression or enhanced cancer cell adhesion to extracellular matrices has been reported to induce PTX-resistance (1,2). Morphological changes in proliferating cancer cells may affect cellular PTX-sensitivity (3). Cancer cells with higher Fas antigen, an apoptosis-inducing cytokine receptor, are more sensitive to PTX than cells with lower Fas expression (4). Stimulation of CD40, a cell survival cytokine receptor, can inhibit PTX-apoptosis (4). Increased expressions of BCL-2 or BCL-XL, which are apoptosis-inhibitory regulators, have been reported to induce PTX-resistance in cancer cells (5). Increased expression of MDR-1, a multi-drug resistance molecule, has been found to be associated with PTX-resistance (6). Chromosome instability may induce PTX-resistance in cancer cells (7). Previous studies on the mechanisms of PTX-resistance have been performed using various types of human cancer cells including ovarian and breast cancer cells. However, until now there has been no study on PTX-resistance involving human endometrial carcinoma cells.

The purpose of our study was to investigate molecular mechanisms that are associated with acquired PTX-resistance and to explore methods for overcoming PTX-resistance using novel PTX-resistant cell lines derived from human endometrial adenocarcinoma cells. As a result, we have found a novel type of PTX-resistance in cancer cells.

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Materials and methods

Anticancer drugs. All anticancer drugs were kind gifts from pharmaceutical companies. PTX and etoposide (VP16) were provided by Bristol-Myers Squibb Japan Co., Ltd. (Tokyo, Japan). Mitomycin C (MMC) and 5-fluorouracil (5FU) were provided by Kirin-Kyowa-Hakko Co., Ltd. (Tokyo, Japan). Pirarubicin-HCl (THP) was provided by Meiji-Seika Kaisha Ltd. (Osaka, Japan). Cisplatin (CDDP) was provided by Nihon-Kayaku Co., Ltd. (Tokyo, Japan). 4-Hydroxy-cyclophosphamide (4OH-CPM), a major active metabolite of cyclophosphamide, was obtained from Shionogi & Co., Ltd. (Osaka, Japan).

Cell line and culture. The moderately differentiated human endometrial endometrioid adenocarcinoma cell line, HEC-1 (8), was purchased from the JCRB Cell Bank (Japan Collection of Research Bioresources Cell Bank, Tokyo, Japan). All cells were cultured in OPTI-MEM (Invitrogen Corp., Carlsbad, CA, USA) supplemented with 5% fetal calf serum (FCS; Equitech Bio Inc., Ingram, TX, USA), 100 U/ml penicillin (PC), 100 µg/ml streptomycin (SM) and 0.25 µg/ml fungizone (Invitrogen) in 5% CO₂/95% air at 37°C.

Establishment of PTX-resistant subclones from HEC-1 cells. To establish PTX-resistant subclones, HEC-1 cells were cultured with various concentrations of PTX for 3-5 weeks, and the surviving cells were collected. This collection procedure after PTX exposure was repeated six times. Finally, five single cell-derived PTX-resistant subclones, designated PTXr3C, PTXr4B, PTXr6F, PTXr7A and PTXr11B, were established using the limiting dilution method. The monoclonality of each PTX-resistant subclone was also confirmed by chromosome analysis (described below). The establishment of these PTX-resistant subclones took 14 months.

Anticancer drug-sensitivity assays. The effects of anticancer drugs on cell growth were assayed as follows. Cells in the log phase were detached with 0.25% trypsin/1 mM EDTA (Invitrogen) and cultured overnight in 96-well plates (5x10³ cells/well). On day 2, various concentrations of anticancer drugs were added to the cells. On day 4, the numbers of viable cells were evaluated using a cell proliferation assay kit (Dojin Corp., Tokyo, Japan) and expressed as the percentages of viable cells relative to the mean number of viable untreated cells. All experiments were performed four to seven times. Data are shown as the mean ± SD, and comparative data were analyzed using Student's t-test (n=6) and ANOVA.

DNA fragmentation assay. HEC-1 parent cells and PTX-resistant subclones in the log phase were detached with 0.25% trypsin/1 mM EDTA and cultured overnight in culture dishes (3x10⁶ cells/dish) containing OPTI-MEM/5% FCS/PC/SM. On day 2, PTX with a final concentration of 2.5 µg/ml was added to the cells after removal of the floating dead cells. On day 4, genomic DNA was extracted from all cells, including the dead ones, using a SepaGene DNA extraction kit (Sankyo-Junyaku Co., Ltd., Tokyo, Japan) and treated with 100 µg/ml of RNase A (Sigma Chemical Co., St. Louis, MO, USA) in TE buffer (10 mM Tris, pH 8.0, 2 mM EDTA) for 90 min at 37°C to remove any contaminating RNA. Next, approximately 20 µg of the genomic

DNA isolated from 5x10⁵ cells was electrophoresed in a 1.4% agarose gel at 50 V for approximately 2 h, stained with 5 µg/ml of ethidium bromide and visualized using UV fluorescence.

Semiquantitative flow cytometric analysis of cell surface antigens. Cells were detached from culture flasks with 3 mM EDTA in phosphate-buffered saline (PBS), and immunostained as follows. The cells (3x10⁵) were incubated with an excess of the primary antibody for 20 min at 4°C and then washed twice with wash buffer (PBS containing 2% fetal calf serum and 0.1% NaN₃). Next, the cells were incubated with FITC-conjugated goat anti-mouse IgG (H+L) (Dako Japan, Kyoto, Japan) for 20 min at 4°C and washed twice with wash buffer. Finally, the cells were suspended in 200 µl of wash buffer and analyzed using a FACSCalibur™ (Beckman Coulter Japan, Tokyo, Japan). The following primary antibodies were used: mouse anti-human CD29 monoclonal antibody (clone K20: Immunotech, Hampshire, UK); mouse anti-human CD30 monoclonal antibody (clone HRS-4: Immunotech, Marseille, France); mouse anti-human CD40 monoclonal antibody (clone mAb89: Immunotech); mouse anti-human tumor necrosis factor receptor (TNFR) (CD120a) monoclonal antibody (Genzyme, Cambridge, MA, USA); mouse anti-human Fas (CD95) monoclonal IgG (clone UB2: MBL, Nagoya, Japan); mouse anti-human CD49a monoclonal antibody (clone TS2/7: AbD Serotec Ltd., Oxford, UK); mouse anti-human CD49b monoclonal antibody (clone 31H4: Serotec Ltd.); mouse anti-human CD49c monoclonal antibody (clone 11G5: Cymbus Biotech Ltd., Chandlers Ford, UK); mouse anti-human CD49d monoclonal antibody (clone 44H6: Cymbus Biotech Ltd.); mouse anti-human CD49e monoclonal antibody (clone SAM1: Beckman Coulter Japan); and mouse anti-human CD49f monoclonal antibody (clone 4F10: Cymbus Biotech Ltd.).

Semiquantitative flow cytometric analysis of intracellular molecules and multidrug-resistance-related molecules. Flow cytometric analysis of intracellular molecules and multidrug-resistance-related molecules was performed as follows. Cells were detached from culture flasks with 3 mM EDTA in PBS, and then washed with wash buffer (PBS containing 2% fetal calf serum). The cells were fixed with 4% paraformaldehyde in 0.1 M NaH₂PO₄ (pH 7.4) for 10 min on ice. After two washes with wash buffer, the cells were treated with 100 mg/ml digitonin (Sigma Chemical Co.) for 10 min at room temperature. After another two washes, the cells were treated with normal mouse IgG to block non-specific binding sites for 5 min at room temperature, followed by incubation with the primary antibody for 30 min at room temperature and two further washes. Finally, the cells were incubated in FITC-conjugated goat anti-mouse IgG (Gibco BRL, Carlsbad, CA) for 30 min at room temperature, washed twice and suspended in 200 µl of wash buffer for analyses using a FACSCalibur™ (Beckman Coulter Japan). The following primary antibodies were used: mouse anti-human BAX monoclonal antibody (clone 2D2: AbD Serotec Ltd.); mouse anti-human BCL-2 monoclonal antibody (clone 100: AbD Serotec Ltd.); mouse anti-human BCL-XL monoclonal antibody (clone 2H12: Acris Antibodies Inc., San Diego, CA); mouse anti-human multi-drug resistance protein-1 (MDR-1) monoclonal antibody (clone UIC2: Acris Antibodies Inc.); mouse anti-human MDR-related protein (MRP) monoclonal antibody (clone

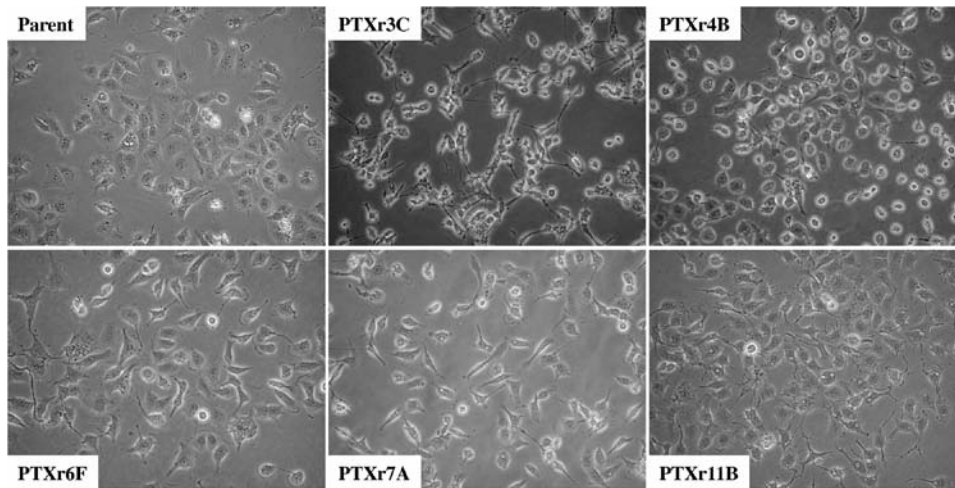


Figure 1. Bright-field microscopic images of cultured HEC-1 parent cells and five PTX-resistant subclones.

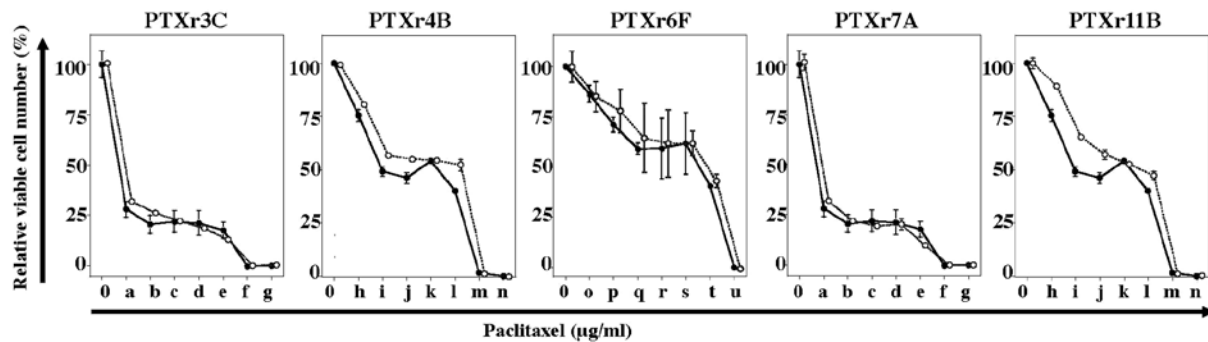


Figure 2. PTX-sensitivity assays of the five PTX-resistant subclones. The solid line with closed circles in each plot is the PTX-sensitivity curve for the parent HEC-1 cells. The dotted lines with open circles are the PTX-sensitivity curves for the PTX-resistant subclones. The final concentrations ($\mu\text{g/ml}$) of PTX indicated as: 0 and a-g at the bottom of the figures were 0, 0.0064, 0.032, 0.16, 0.8, 4, 20 and 100 $\mu\text{g/ml}$; 0 and h-n at the bottom of the figures were 0, 0.0015, 0.0076, 0.0384, 0.192, 0.96, 4.8 and 24 $\mu\text{g/ml}$; and 0 and o-u at the bottom of the figure were 0, 0.00256, 0.0128, 0.064, 0.32, 1.6, 8 and 40 $\mu\text{g/ml}$.

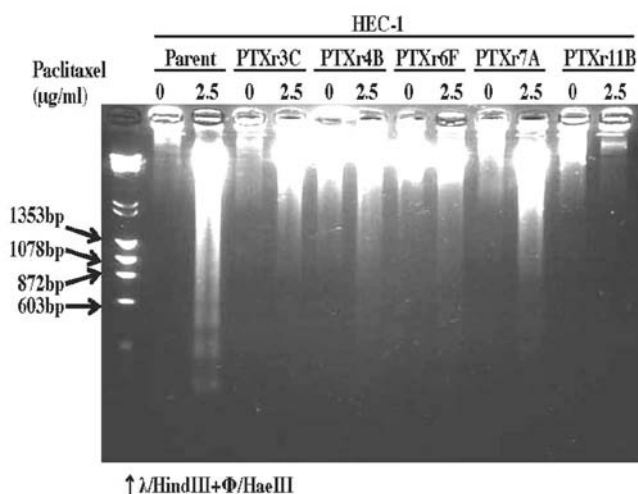


Figure 3. DNA fragmentation assays of the PTX-resistant subclones.

QCLR-1: Merck Millipore, Darmstadt, Germany); and mouse anti-human lung-resistance protein (LRP) monoclonal antibody (clone LRP-56: Merck Millipore).

Karyotyping analysis. Cytogenetic analysis was performed according to previously reported methods (9,10) with the following modifications. Briefly, tumor cell cultures were washed and incubated with 0.1% (v/v) colcemide (Sigma Chemical Co.) overnight. The cells were then exposed to a hypotonic solution composed of 3 g/l KCl, 0.2 g/l EGTA and 4.8 g/l Hepes. The cells were centrifuged into a pellet and fixed in a methanol solution. G-banding was performed using the method of Yunis (11).

Results

Establishment of monoclonal PTX-resistant subclones. Five monoclonal PTX-resistant subclones, PTXr3C, PTXr4B, PTXr6F, PTXr7A and PTXr11B, were independently established from HEC-1 cells using long-term PTX-exposure cultures and limiting dilution cultures. Under the microscope, their cellular appearance and adherence to the culture dishes could not be discriminated from those of the parent HEC-1 cells (Fig. 1). Although PTX-sensitivity tests of the PTX-resistant subclones were performed 4-7 times for each subclone, all of the five PTX-resistant subclones showed no significant change in PTX-induced growth inhibition as compared with the HEC-1 parent cells (Fig. 2). However, it

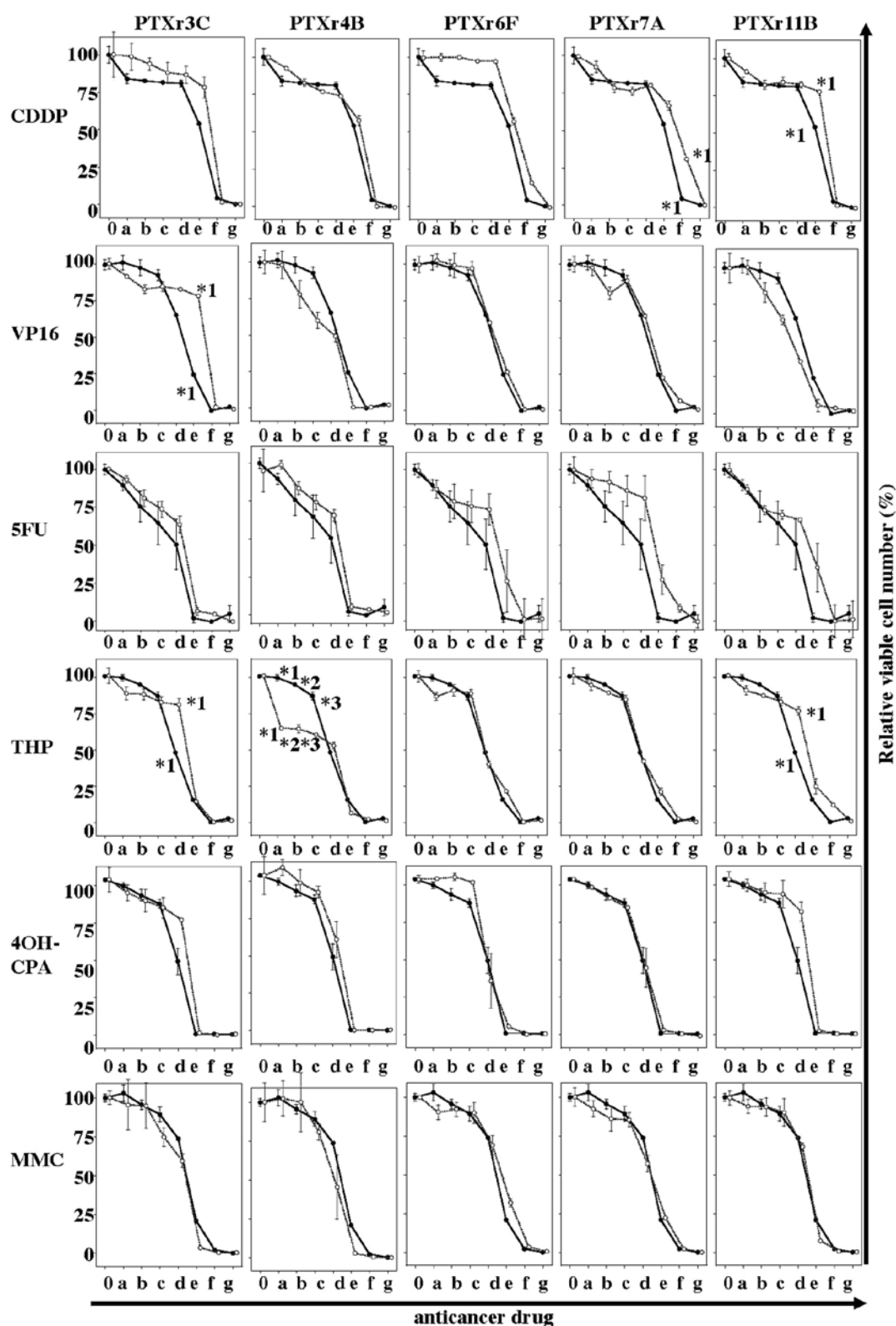


Figure 4. Anticancer drug-sensitivity assays of the five PTX-resistant subclones. The solid line with closed circles in each plot is the drug-sensitivity curve for the parent HEC-1 cells. The dotted lines with open circles are the drug-sensitivity curves for the PTX-resistant subclones. The final concentrations ($\mu\text{g/ml}$) of each anticancer drug indicated as 0 and a-g at the bottom of the figures were as follows: CDDP, 0, 0.032, 0.16, 0.8, 4, 20, 100 and 500 $\mu\text{g/ml}$; VP16, 0, 0.256, 1.28, 6.4, 32, 160, 800 and 4,000 $\mu\text{g/ml}$; 5FU, 0, 0.32, 1.6, 8, 40, 200, 1,000 and 5,000 $\mu\text{g/ml}$; THP, 0, 0.001067, 0.00533, 0.0267, 0.133, 0.67, 3.33 and 16.670 $\mu\text{g/ml}$; 4OH-CPA, 0, 0.32, 1.6, 8, 40, 200, 1,000 and 5,000 $\mu\text{g/ml}$; and MMC, 0, 0.128, 0.064, 0.32, 1.6, 3.2, 40 and 200 $\mu\text{g/ml}$. *1-3, $p < 0.05$.

was demonstrated using the DNA fragmentation assay that these five PTX-resistant subclones were apparently more resis-

tant to PTX-induced DNA fragmentation, or in other words PTX-induced apoptosis, than the parent HEC-1 cells (Fig. 3).

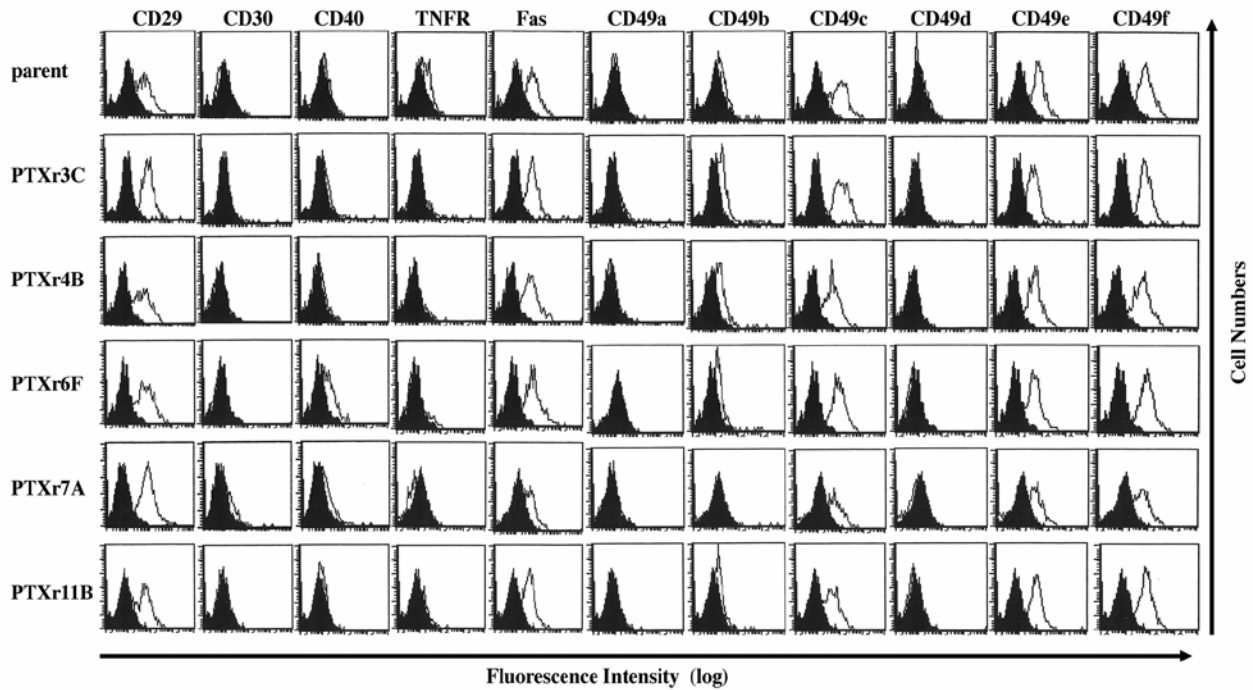


Figure 5. Semiquantitative flow cytometric analyses of the expression of cell surface antigen on the PTX-resistant subclones. In the panels, the black histograms show the negative controls, and the white histograms show the positive reactions.

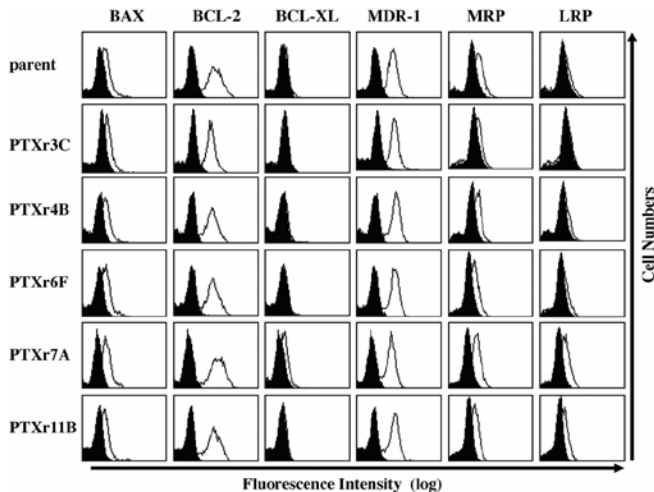


Figure 6. Semiquantitative flow cytometric analyses of the expression of intracellular apoptosis-regulating molecules and multidrug-resistance-related molecules in the PTX-resistant subclones. In the panels, the black histograms show the negative controls and the white histograms show the positive reactions.

Anticancer drug-sensitivities of the PTX-resistant subclones. Anticancer drug-sensitivity assays against six anticancer drugs were performed on the PTX-resistant cell lines. As shown in Fig. 4, all the drug-sensitivity curves for the five PTX-resistant subclones showed very similar results to those for the HEC-1 parent cells. There were no PTX-resistant subclones that had moderate or high cross-resistance to any other anticancer drug.

Flow cytometric analysis of cell surface antigen expressions in the PTX-resistant cells. The cell surface antigen expression

profiles of the PTX-resistant cell lines were investigated using semi-quantitative flow cytometric analysis. Because all of the PTX-resistant subclones showed resistance to PTX-induced DNA fragmentation, we examined the 11 cell surface antigens that were reported to affect cell apoptosis or cell survival. The flow cytometric data from the parent HEC-1 cells were compared with those from the PTX-resistant cells. However, there were no apparent differences among the flow cytometric profiles of the parent HEC-1 cells and the five PTX-resistant cell lines (Fig. 5).

Semiquantitative flow cytometric analysis of intracellular molecules and multidrug-resistance-related molecules. To investigate the mechanisms of acquired resistance to PTX-induced apoptosis in the PTX-resistant subclones, semiquantitative flow cytometric analysis of three intracellular apoptosis-regulating molecules and three multidrug-resistance-related molecules was performed. As shown in Fig. 6, no apparent differences in the expression of the six molecules were found among the flow cytometric profiles of the HEC-1 parent cells and the five PTX-resistant cell lines.

Karyotyping analysis of the PTX-resistant cells. Karyotyping analyses of the five *in vitro*-cultured PTX-resistant subclones were examined (Fig. 7). The karyotype of the parent HEC-1 cell line was 74, XX, i(X)(p10), +1, add(1)(p36)x2, der(1)t(1;12)(p22;q11), -2, add(2)(q23)x2, add(3)(p21), -4, +5, -6, add(6)(q21), +7, -8, +add(9)(p11), i(9)(q10), add(12)(q24), der(12)t(12;14)(p13;q13), -13, -14, add(15)(p11), +16, +add(17)(p11), add(17)(p11), -18, -19, -20, +21, add(21)(p11)x2, -22, +8mar (Fig. 7A). The karyotype of the PTXr3C cell line was 75, XX, i(X)(p10), add(1)(p36), der(1)t(1;12)(p22;q11), -2, add(2)(q23)x2, add(3)



Figure 7. Karyotyping analysis of HEC-1 parent cells and HEC-1-derived PTX-resistant subclones.

(p21), +5, -6, add(6)(q21), +7, -8, +add(9)(p11), +11, -12, add(12)(q24), der(12)t(12;14)(p13;q13), -13, -14, add(15)(p11), +add(17)(p11), add(17)(p11), add(18)(q23), -19, add(21)(p11)x2, -22, +9 mar (Fig. 7B). The karyotype of the PTXr4B cell line was 75, XX, i(X)(p10), add(1)(p36), der(1)t(1;12)(p22;q11), -2, add(2)(q23)x2, add(3)(p21), +5, -6, add(6)(q23), +add(7)(q32), -8, add(9)(p11), +11, -12, add(12)(q24), der(12)t(12;14)(p13;q13), -13, -14, add(15)(p11), +add(17)(p11)x2, add(17)(p11), add(18)(q23), -19, add(21)(p11)x2, -22, +9 mar (Fig. 7C). The karyotype of the PTXr6F cell line was 77, XX, i(X)(p10), add(1)(p36), der(1)t(1;12)(p22;q11), -2, add(2)(q23)x2, -3, add(3)(p21), +5, -6, add(6)(q23), +7, +add(9)(p11), -10, +11, -12, add(12)(q24), der(12)t(12;14)(p13;q13), -13, -14, add(15)(p11), +add(17)(p11), add(17)(p11), add(18)(q23), -19, +21, add(21)(p11)x3, -22, +11

mar (Fig. 7D). The karyotype of the PTXr7A cell line was 78, XX, i(X)(p10), add(1)(p36), der(1)t(1;12)(p22;q11), -2, add(2)(q23)x2, add(3)(p21), +5, -6, add(6)(q23), +7, -8, +add(9)(p11), +11, add(12)(q24), der(12)t(12;14)(p13;q13), -13, -14, add(15)(p11), +add(17)(p11), add(17)(p11), add(18)(q23), -19, -20, add(21)(p11)x2, -22, +11 mar (Fig. 7E). The karyotype of the PTXr11B cell line was 76, XX, i(X)(p10), add(1)(p36), der(1)t(1;12)(p22;q11), -2, add(2)(q23)x2, add(3)(p21), -6, add(6)(q23), +7, -8, +add(9)(p11), add(12)(q24), der(12)t(12;14)(p13;q13), -14, add(15)(p11), +add(17)(p11), add(17)(p11)x2, add(18)(q23), -19, add(21)(p11)x2, -22, +9 mar (Fig. 7F). As a result, common chromosomal abnormalities were found in chromosome 4 and chromosome 18 among the five PTX-resistant subclones, but not in parent HEC-1 cells.

Discussion

PTX stabilizes microtubules and, as a result, interferes with the normal breakdown of microtubules during cell division. Therefore, PTX-treated cells have defects in the mitotic spindle assembly, chromosome segregation and cell division. Although PTX is now widely used to treat cancer, acquired or natural PTX-resistance is a major clinical problem in cancer chemotherapy. In order to investigate how to overcome acquired PTX-resistance, we have established five novel monoclonal PTX-resistant subclones from HEC-1, a human endometrioid adenocarcinoma cell line. This is probably the first study in which PTX-resistant cells from human endometrial cancer cells have been established. Most interestingly, all of the established PTX-resistant cell lines showed resistance to PTX-induced DNA fragmentation, but not to PTX-induced growth suppression. The differential resistance exhibited by the PTX-resistant cell lines may be a very rare phenomenon. We could not find any reports concerning anticancer drug-resistant cancer cell lines with such differential resistance as that shown by the PTX-resistant cell lines.

The PTX-resistant cell lines showed no significant changes in sensitivity to drug-induced growth suppression, not only by PTX but also by the six other anticancer drugs used in our study. Using the same experimental methods as those used to establish the present PTX-resistant cell lines, we have previously established many monoclonal anticancer drug-resistant cell lines such as CPA-resistant subclones (12), 5FU-resistant subclones (13), CDDP-resistant subclones (14) and VP16-resistant subclones (15). Almost all of these drug-resistant subclones demonstrated cross-resistance to several anticancer drugs including PTX. Both CPA-resistant subclones (12) and 5FU-resistant subclones (13) exhibited IC_{50} s for PTX-sensitivity that were >125 times higher than those of their parent cell lines, while VP16-resistant subclones (15) had IC_{50} s for PTX-sensitivity that were up to five times higher than those of their parent cell lines. The CDDP-resistant subclones had IC_{50} s that were approximately 25 times higher than their parent cell line (14). All of the five PTX-resistant subclones established in the present study showed no apparent cross-resistance to six other anticancer drugs. Most interestingly, the PTX-resistant subclones showed almost the same resistance to 4-OH-CPA and 5FU as the parent HEC-1 cells or very weak cross-resistance to 4-OH-CPA and 5FU. These results suggest that after PTX-chemotherapy PTX-resistant cancer cells may retain high sensitivities to other anticancer drugs. On the other hand, after chemotherapy with CPA, 5FU or CDDP drug-resistant cancer cells might have acquired strong cross-resistance to PTX simultaneously.

All five PTX-resistant subclones showed acquired resistance to PTX-induced DNA fragmentation (PTX-induced apoptosis) but not to PTX-induced growth suppression. These results indicate the possibility that PTX-induced apoptosis is regulated by different intracellular mechanisms from PTX-induced growth suppression. For example, strong cross-resistance to PTX-induced growth suppression was found in CPA-resistant subclones derived from the cervical carcinoma cell line ME180 (12) and 5FU-resistant subclones derived from the endometrial carcinoma cell line HHUA (13), while little cross-resistance to PTX-induced growth suppression was found in VP16-resistant ME180 cells (15). Therefore, the differential

effects between PTX-induced growth suppression signals and PTX-induced apoptosis signals may depend on the anticancer drugs that were used to establish the drug-resistant cell lines.

The PTX-sensitivity of cancer cells can be regulated by morphological changes in proliferating cancer cells (3) while there was no apparent difference in microscopic findings between the established PTX-resistant cells and parent cells. Morphological changes in proliferating cancer cells are affected by the expression levels and functions of cell adhesion molecules in the cells. In fact there are several reports that increased β 1-integrin expression or increased cell adhesion to extracellular matrices induced resistance to PTX (1,2,17). Another study demonstrated increased integrin expression in PTX-resistant cells (18). The VP16-resistant subclones, which have weak cross-resistance to PTX, showed decreased expression of CD29, CD49a and CD49f (15). However, the five PTX-resistant subclones established in our study did not show any significant differences in CD29 and CD49a-f expression profiles from those of the parent HEC-1 cells. Because there was no difference in integrin expression and cell proliferation patterns between PTX-resistant cells and the parent cells, the mechanisms of PTX-resistance in the established PTX-resistant subclones may not be caused by the differential cell adhesion ability of the PTX-resistant cells.

Both Fas and TNFR are well-known receptors for apoptosis-inducing cytokines. There are several reports that PTX sensitivity can be regulated by apoptosis signals via Fas or TNFR. The high Fas-expressing cells are reported to be more sensitive to PTX (4). PTX treatment can increase production of TNF α (19) and Fas ligand (20) to induce apoptosis. On the other hand, PTX can inhibit TNF α functions in corneal endothelium (21). CD40 is a well-known receptor for the cell survival cytokine, CD40L. Increased CD40 expression has been reported in VP16-resistant cells (15). CD40-stimulation may induce anticancer multidrug-resistance in cancer cells (22) and inhibit PTX-induced apoptosis (4). However, in the PTX-resistant subclones in the present study, there was no significant change in the expression of Fas, TNFR and CD40 between PTX-resistant cells and the parent cells.

BCL-2 family products are well-known apoptosis-regulators. There are several reports that increased BCL-2 expression and/or BCL-XL expression can induce PTX-resistance (5,23,24). Decreased BAX expression has also been reported to be related to PTX-resistance (25,26). However, in the PTX-resistant subclones in our study there was no significant change in the expression of BCL-2, BCL-XL and BAX between PTX-resistant cells and the parent cells.

MDR-1, MRP and LRP have been identified as multidrug-resistance-related molecules. Several studies have found that PTX-resistant cells had increased MDR-1 expression (6) or LRP expression (27), although MRP expression may not have any relationship to PTX-resistance (5,6). In the PTX-resistant subclones in the present study, there was no significant change in the expression of MDR-1, MRP and LRP between PTX-resistant cells and the parent cells.

Because chromosome instability has been reported to affect PTX-resistance (7), we performed karyotype analyses of the established PTX-resistant subclones. All of the five PTX-resistant subclones had common changes in chromosomes 4 and 18 that were different from the parent cells. This finding contrasts with a study involving 5FU-resistant cells, which reported that there

were no apparent changes in karyotypes relative to the parent cells (13). Chromosomes 4 or 8 might have key genes that affect sensitivity to PTX-induced apoptosis.

Although we have established novel monoclonal PTX-resistant subclones from human endometrial cancer cells, the mechanisms involved in PTX-resistance remain unclear. Most notably, these subclones showed resistance to PTX-induced apoptosis but not to PTX-induced growth suppression. During the acquisition process of PTX-resistance, large differences occurred in the PTX-concentrations required for the induction of growth suppression and apoptosis. In the present study, a much higher dose of PTX was necessary to induce apoptosis in PTX-resistant cells than to induce growth suppression. As shown in Fig. 2, the PTX growth-inhibition curves are stair-like (12,13,14), while those for the other anticancer drugs are sigmoid. Since the docetaxel growth-inhibition curves are also stair-like (15,28), differential sensitivity to drug-induced apoptosis and drug-induced growth suppression might be a phenomenon specific to taxane compounds. In conclusion, the novel PTX-resistant subclones from HEC-1 established in our study can be used as experimental models for recurrent cancers occurring after complete remission as a result of PTX-chemotherapy. They may prove to be very useful tools for the investigation of methods to prevent or treat recurrent cancers after PTX-chemotherapy.

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