# Establishment and Characterization of Multidrug-resistant Gastric Cancer Cell Lines

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**Abstract.** *Aim: The aim of this study was to establish drug-resistant cell lines and to elucidate mechanisms leading to multi-drug resistance in gastric cancer. Materials and Methods: Five cancer cell lines resistant to 5-fluorouracil, paclitaxel, oxaliplatin, irinotecan, or gemcitabine, were respectively established from a parent gastric cancer cell line, OCUM-2M, by stepwise exposure to each chemotherapeutical agent. Results: Cell death by apoptosis induced by anti-cancer drugs was low in 5 chemo-resistant cell lines. Percentage of cells in S and $G_0/G_1$ phase was low in cell lines resistant to oxaliplatin or irinotecan. Cell lines resistant to paclitaxel, oxaliplatin, and gemcitabine showed multi-drug resistance. Alterations in MRP, DAPK1, or DAPK2 expression were found in multi-drug resistant cell lines. Conclusion: The cell-cycle distribution and alterations of MRP, DAPK1, and DAPK2 genes may be integral part of mechanisms responsible for chemo-resistance. These cell lines might be useful to study molecular mechanisms leading to multi-drug resistance.*

Gastric cancer remains one of the major causes of cancer death around the world (1), and most patients in the advanced stages of cancer therefore require chemotherapy. One of the greatest obstacles to effective chemotherapy is the development of drug resistance. In addition, drug resistant cancer cells sometimes develop cross-resistance to a wide variety of chemotherapeutic drugs, a phenomenon known as multi-drug resistance (MDR). The acquisition of MDR makes the determination of second-line therapy even more difficult and is a major cause of gastric cancer treatment failure (2). However, there are not definitive findings that indicate the best strategy for selecting the suitable agents for chemo-resistant tumors (3). It is important to determine the appropriate second-line chemotherapeutical agent to use against chemo-resistant cancers (4). 5-Fluorouracil (5FU) is one of the most effective anticancer drugs used in the chemotherapy of gastric cancer for decades (5). Paclitaxel (PTX), oxaliplatin (OXA), and Irinotecan (SN38) are recently emergent drugs (6). Gemcitabine (GEM), a pyrimidine analog, is widely applied in lung cancer and pancreatic cancer. GEM is not widely used in gastric cancer, but it is considered to be an effective agent for this type of cancer (7, 8). The administration of these anticancer drugs has improved the efficacy of chemotherapy in gastric cancer, however most tumors acquire chemo-resistance after undergoing consecutive treatments.

The use of chemo-resistant cancer cell lines might be one of useful model systems to study molecular mechanisms leading to anticancer drug resistance. However, there have so far been few reports on the establishment of gastric cancer cell lines resistant to multi-chemotherapeutic drugs, and the analysis of the cross-resistance to other anticancer drugs (9, 10).

The aim of this study was to establish drug-resistant cell lines *in vitro* and to elucidate molecular mechanisms leading to multi-drug resistance in gastric cancer.

## Materials and Methods

*Anti-cancer drugs.* Five anti-cancer drugs, 5FU (Kyowa Hakko, Tokyo, Japan), PTX (Bristol-Myers, Wallingford, Connecticut), OXA (Yakult, Tokyo, Japan), irinotecan active metabolite SN38 (Yakult) and GEM (Eli Lilly, Kobe, Japan), were used in this study. All reagents were used as recommended by their suppliers.

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*Key Words:* Chemotherapy, cross-resistance, gastric cancer, anticancer drugs, apoptosis, cell-cycle.

*Establishment of anti-cancer drugs resistant cancer cell lines*. The human gastric cancer cell line, OCUM-2M, was used in this study. OCUM-2M was established from a diffuse type of primary gastric cancer obtained during a total gastrectomy (11). No chemotherapy was performed before the specimen was resected. OCUM-2M cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Nikken Biomedical Laboratory, Kyoto, Japan). The media were supplemented with 10% fetal bovine serum, 100 IU/ml penicillin (ICN Biomedical, Costa Mesa, CA), 100 μg/ml streptomycin (ICN Biomedical), and 0.5 mM sodium pyruvate (Cambrex, Walkersville, MD, USA). The cells were cultured at 37˚C in a humidified atmosphere of 5% $CO_2$ in air. Anticancer-drugs resistant cells were established by exposure to increasing concentrations of 5 anticancer drugs, 5FU, PTX, OXA, SN38 or GEM similar to the previously described method (12). OCUM-2M cells were initially cultured in DMEM containing 5FU, PTX, OXA, SN38 or GEM each drug alone at the concentration of 1/160 of their 50% growth inhibition ($IC_{50}$), and then the cells were subcultured every 2 weeks in DMEM with increased concentrations of anticancer drugs, a 25% increase each time. Finally, the resultant cell lines that grew exponentially in the presence of high concentrations were designated as drug resistant gastric cancer cell lines, and named OCUM-2M/5FU, OCUM-2M/PTX, OCUM-2M/OXA, OCUM-2M/SN38 and OCUM-2M/GEM respectively. The final drug concentrations that were tolerated by each cell line are shown in Table I. Each cell line proliferated when exposed to an anticancer drug at a concentration between 0.2 and 2 times of their $IC_{50}$. In all resistant cell lines, experiments were performed after culturing for at least 4 weeks in the absence of the anti-cancer drugs.

*Growth-inhibition assay*. The effect of anti-cancer drugs on OCUM-2M cell viability was determined using a 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Wako, Osaka, Japan) colorimetric assay. Cancer cells ($2.5\times10^4$/ml) were seeded into a 96-well plate in DMEM containing different concentrations of each anti-cancer drug. After incubation for 72 hr at 37˚C, 20 μl of MTT (5 mg/ml in PBS) were added to each well and the plates were incubated at 37˚C for 3 hr, and then 200 ml of dimethyl sulfoxide (Wako) was added. The formazan product of MTT was measured as the absorbance at 550 nm using a microtiter plate reader (Model 550; Bio-Rad Laboratories, Tokyo, Japan). The percentage of cell viability was determined as the ratio of the absorbance of the sample versus the control. The $IC_{50}$ was determined as the drug concentration showing 50% cell growth inhibition in comparison to the control cell growth. The resistance index (RI) was determined as the ratio of the $IC_{50}$ of the drug resistant cell line/$IC_{50}$ of OCUM-2M. Chemo-resistant cancer cell line was determined when RI of cancer cells showed 3 or more. Six replicate wells were used for each drug concentration, and the testing was carried out 3 times independently.

*Detection of cross-resistance to anti-cancer drugs*. Either OCUM-2M or the 5 drug resistant sublines were seeded into 96-well plates with or without the addition of each of the anticancer drugs at concentration of their $IC_{50}$ of OCUM-2M, and the plates were incubated for 72 hr at 37˚C. The suppression of cell proliferation was examined using the MTT assay. The $IC_{50}$ of the 5 sublines to each drug was calculated using the methods described above. Six replicate wells were used for each drug concentration, and the testing was carried out 3 times independently.

Table I. *The drug concentration tolerated by each drug resistant subline from OCUM-2M*.

| | OCUM-2M subline | |
|---|---|---|
| Anti cancer drug | Drug resistant cell line | Drug concentrations ($IC_{50}$[a], ratio) |
| 5FU | OCUM-2M/5FU | 1.05 μM (=0.2×$IC_{50}$) |
| PTX | OCUM-2M/PTX | 7.1 nM (=1.5×$IC_{50}$) |
| OXA | OCUM-2M/OXA | 2.35 μM (=0.5×$IC_{50}$) |
| SN38 | OCUM-2M/SN38 | 4.5 nM (=0.5×$IC_{50}$) |
| GEM | OCUM-2M/GEM | 366 nM (=2×$IC_{50}$) |

[a]The $IC_{50}$ of OCUM-2M to 5FU, PTX, OXA, SN38 and GEM was 5.1 μM, 4.7 nM, 4.7 μM, 9.0 nM and 183 nM, respectively. $IC_{50}$, 50% growth inhibition.

*Apoptosis assay*. Apoptosis was detected using flow cytometry by staining cells with annexin V-FITC and propidium iodide (Medical & Biological Laboratories CO., LTD, Nagoya, Japan). Cancer cells were seeded at a density of $1.0\times10^5$ cells/ml in a 6-well plate. With or without anti-cancer drugs at the concentration of $IC_{50}$, the plates were incubated for 72 h at 37˚C. Using Apoptosis Kit, cells were stained with Annexin V-FITC and propidium iodide according to the instructions of the supplier, incubated for 15 min at room temperature in the dark, and immediately analyzed by FACScan flow cytometry (Becton Dickinson, Mountain View, CA).

*Cell cycle test*. Cancer cells ($2\times10^4$ cells) were harvested and managed according to instructions of the Cycle TEST PLUS DNA reagent kit protocol (Becton Dickinson, Mountain View, CA, USA), then incubated with ribonuclease A for 10 min at room temperature, and with propidium iodide for 30 min in the dark on ice. The sub-$G_0/G_1$, S, and $G_2$/M phase fractions of $2\times10^4$ cells were determined by flow cytometry using a FACScaliber (Becton Dickinson). The results were analyzed using the Modofit software program (Beckton Dickinson).

*Reverse transcription PCR*. The mRNA expression of genes, including *MDR1, MRP, DAPK1, DAPK2, DAPK3, DPD, TK1, TS, TP, MAD2L1, SLC28A3, HMGB1, RRM1, UMPK, TRAG3, Caspase3*, and *P53*, by reverse transcription PCR (RT-PCR). Total cellular RNA was extracted from OCUM-2M and the 5 drug resistant cell lines using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After the genomic DNA was removed by DNase, cDNA was prepared from 1 μg of RNA with Maloney mouse leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA) using random primers (Invitrogen). The relevant cDNA were amplified by PCR using specific primer pairs (Table II) with Taq DNA polymerase (Invitrogen) in a thermal cycler. The PCR conditions were as follows: pre-denaturation at 94˚C for 3 min, denaturation at 94˚C for 30 sec, annealing at 55˚C for 30 sec, extension at 72˚C for 1 min with 35 cycles of the three repeated steps, and final incubation at 72˚C for 10 min. The PCR products were separated by electrophoresis on a 2% agarose gel. The mRNA expression of the each gene was normalized to that of the housekeeper *GAPDH* gene.

*Statistical methods*. The values of different groups were compared using Student's *t*-test. Probability values of $p<0.05$ were regarded as statistically significant. All statistical tests were two-sided.

Table II. *Primer sequences and size of PCR products*.

| Genes | | Sequences | Size of PCR products |
|---|---|---|---|
| *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* | sense | ACCTGACCTGCCGTCTAGAA | 247bp |
| | antisense | TCCACCACCCTGTTGCTGTA | |
| *multi-drug resistance related genes multidrug resistance 1 (MDR1)* | sense | GCCATAGCTCGTGCCCTTGT | 212bp |
| | antisense | CCATGCTCCTTGACTCTGCC | |
| *multidrug resistance protein (MRP)* | sense | GGGCTTCTCTGTCTCTGCTG | 285bp |
| | antisense | ATGCCCACCTTCTCTCCTGC | |
| *death-associated protein kinases 1 (DAPK 1)* | sense | TCTACCAGCCACGGGACTTC | 134bp |
| | antisense | GCTGGCCTGTGAGTAGACGT | |
| *death-associated protein kinases 2 (DAPK 2)* | sense | GCATCGTGTCCCTGTGCAAC | 121bp |
| | antisense | GCTTTCCTCCTGGCGATGTC | |
| *death-associated protein kinases 3 (DAPK 3)* | sense | CCCAACCCACGAATCAAGCTC | 236bp |
| | antisense | GCTGAGATGTTGGTGAGCGTC | |
| *dihydropyrimidine dehydrogenase (DPD)* | sense | TGACCTCCATTGCTCGTGCTC | 275bp |
| | antisense | GCTTTGAGGCCAGTGCAGTAG | |
| *thymidine kinase 1 (TK1)* | sense | CTGCACTGGATGGGACCTTC | 164bp |
| | antisense | CCCAATCACCTCGACCTCCT | |
| *thymidylate synthase (TS)* | sense | GTGGAGGCATTTTGGGGCAG | 265bp |
| | antisense | GTTGAAAGGCACACCGAGGC | |
| *solute carrier family 28 member 3 (SLC28A3)* | sense | CTTCCTGGCCCTGCTGTCTT | 140bp |
| | antisense | CCTGCCATTCCACTCCCATC | |
| *mitotic arrest deficient, homolog-like 1 (MAD2L1)* | sense | AGCTACGGTGACATTTCTGCCA | 198bp |
| | antisense | GGAATTTTGTAGGCCACCATGC | |
| *thymidine phosphorylase (TP)* | sense | CTGAGCGAAGCGGACATCAG | 111bp |
| | antisense | CAGATCCATGCCCCGAAGTC | |
| *high-mobility group box 1 (HMGB1)* | sense | GGCCTTCTTCCTCTTCTGCT | 175bp |
| | antisense | CCTCATCTTCCTCCTCTTCC | |
| *ribonucleotide reductase M1 polypeptide (RRM1)* | sense | GGATGAGGTTTGGGGAGAGGAA | 182bp |
| | antisense | GGTTCTGCTGGTTGCTCTTTCG | |
| *uridine monophosphate kinase (UMPK)* | sense | GGCTACACACACCTTTCTGCA | 335bp |
| | antisense | CCACTACTCTTTCCCCTCTCA | |
| *paclitaxel resistance associated gene 3 (TRAG3)* | sense | GAGTTTCATGCCTGCTGG | 161bp |
| | antisense | CTCTTTGGTGTTGGTGGGT | |
| *Caspase 3* | sense | GGCATTGAGACAGACAGTGGTG | 152bp |
| | antisense | GCACAAAGCGACTGGATGAACC | |
| *P53* | sense | AGCGATGGTCTGGCCCCTCCT | 120bp |
| | antisense | CTCAGGCGGCTCATAGGGCAC | |

## Results

*Establishment of chemo-resistant gastric cancer cell lines*. The $IC_{50}$ of OCUM-2M and 5 resistant cell lines are shown in Table III. The $IC_{50}$ of OCUM-2M to 5FU, PTX, OXA, SN38 and GEM was 5.1 μM, 4.7 nM, 4.7 μM, 9.0 nM and 183 nM, respectively. In contrast, the $IC_{50}$ of the 5 resistant gastric cancer cell lines, OCUM-2M/5FU, OCUM-2M/PTX, OCUM-2M/OXA, OCUM-2M/SN38, and OCUM-2M/GEM, was 55 μM, 15 nM, 86 μM, 567 nM and 587 nM against 5FU, PTX, OXA, SN38, and GEM, respectively. The resistance index (RI) was determined as the ratio of the $IC_{50}$ of the drug resistant cell line/$IC_{50}$ of parent OCUM-2M (Figure 1). RI of OCUM-2M/5FU, OCUM-2M/PTX, OCUM-2M/OXA, OCUM-2M/SN38, and OCUM-2M/GEM cells against 5FU, PTX, OXA, SN38, and GEM were 10.8, 3.2, 18.3, 63, and 3.2, respectively. Since all of the RI of the 5 cell lines were more than 3.0, these cell lines were successfully established as chemo-resistant cancer cell lines. No apparent morphologic difference was found in each drug resistant cell lines. Figure 2 shows the cytotoxic effects of various anticancer drugs against

Table III. *$IC_{50}$ of OCUM-2M and the drug resistant cell lines*.

| Cell line | 5FU (μM) | PTX (nM) | OXA (μM) | SN38 (nM) | GEM (nM) |
|---|---|---|---|---|---|
| OCUM-2M (control) | 5.1 | 4.7 | 4.7 | 9 | 183 |
| OCUM-2M/5FU | 55.0 | 6.5 | 3.9 | 13.8 | 271 |
| OCUM-2M/PTX | 15.9 | 15.1 | 20.4 | 63.2 | 397 |
| OCUM-2M/OXA | 11.3 | 17.3 | 86.2 | 18.4 | 300 |
| OCUM-2M/SN38 | 7.1 | 2.8 | 5.1 | 567 | 267 |
| OCUM-2M/GEM | 19.5 | 5.5 | 7.4 | 15.5 | 587 |

$IC_{50}$, 50% growth inhibition.

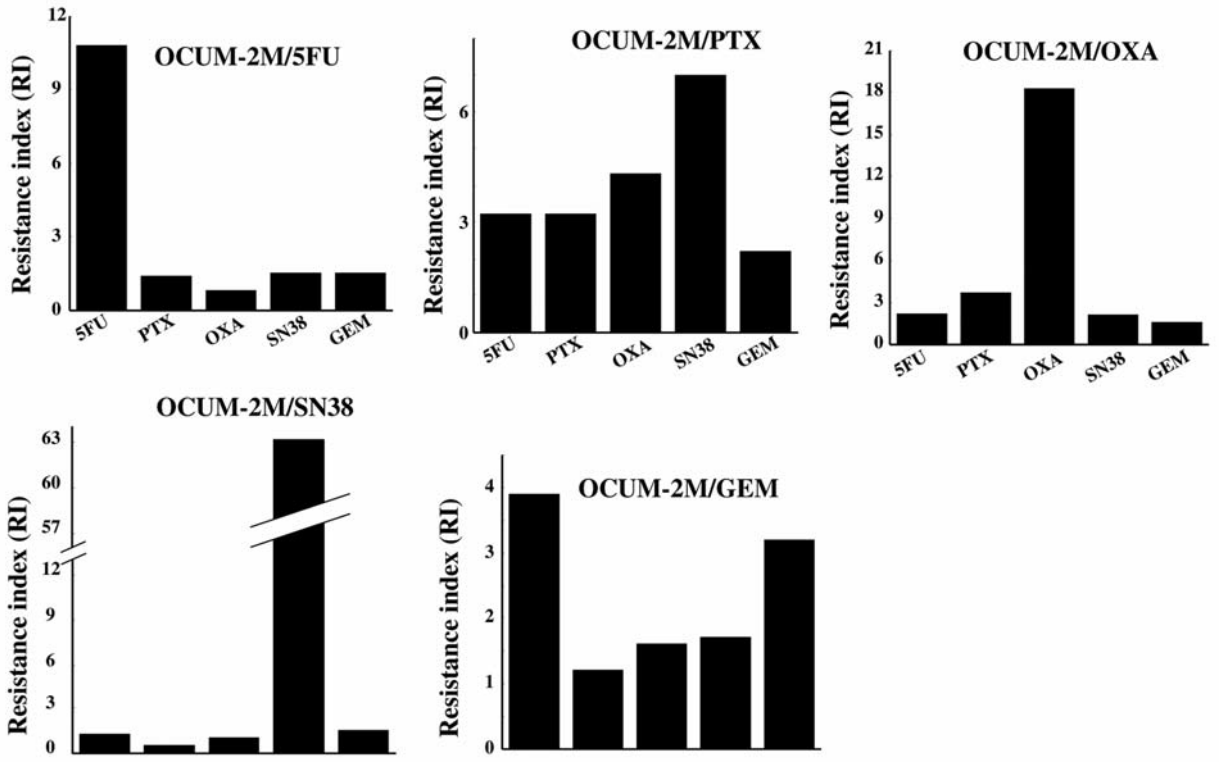


Figure 1. *Resistance index of drug resistant cell lines. The resistance index (RI) was determined as the ratio of the $IC_{50}$ of the drug resistant cell line/IC50 of parent OCUM-2M. RI of OCUM-2M/5FU against 5FU, PTX, OXA, SN38, and GEM were 10.8, 1.4, 0.8, 1.5 and 1.5, respectively, RI of OCUM-2M/PTX is 3.2, 3.2, 4.3, 7.0 and 2.2, RI of OCUM-2M/OXA is 2.2, 3.7, 2.1, 18.3 and 1.6, RI of OCUM-2M/SN38 is 1.4, 0.6, 1.1, 63 and 1.5, and RI of OCUM-2M/GEM is 3.9, 1.2, 1.6, 1.7 and 3.2. RI of OCUM-2M/PTX, OCUM-2M/OXA, and OCUM-2M/GEM cells was over 3.0 in more than 2 drugs. In contrast, RI of OCUM-2M/5FU or OCUM-2M/SN38 cells was over 3.0 in 5FU or SN38 alone.*

the parent and resistant cell lines. The proliferation of OCUM-2M cells following the addition of $IC_{50}$ of 5FU, PTX, OXA, SN38 and GEM was 59.6%, 46.2%, 54.3%, 53.3% and 53.2%, respectively. In contrast, the proliferation of OCUM-2M/5FU, OCUM-2M/PTX, OCUM-2M/OXA, OCUM-2M/SN38, and OCUM-2M/GEM cells when exposed to 5FU, PTX, OXA, SN38 and GEM at a concentration of $IC_{50}$ for OCUM-2M cells were significantly ($p<0.01$) high, compared with that of OCUM-2M cells (Figure 2).

*Cross-resistance to other anti-cancer drugs*. The RI of OCUM-2M/5FU and OCUM-2M/SN38 to anticancer drugs was less than 3.0, except 5FU and SN38, respectively. In contrast, the RI of OCUM-2M/PTX to 5FU, PTX, OXA, and SN38 is over 3.0. The RI of OCUM-2M/OXA to PTX and OXA is over 3.0. The RI of OCUM-2M/GEM to 5FU and GEM is over 3.0. These findings suggested that OCUM-2M/PTX, OCUM-2M/OXA, and OCUM-2M/GEM cells showed cross-resistance to other anticancer drugs, but not OCUM-2M/5FU and OCUM-2M/SN38 cells (Figure 1). 5FU decreased the proliferation of OCUM-2M/OXA and OCUM-2M/SN38 cells. PTX and OXA decreased the proliferation of OCUM-2M/5FU, OCUM-2M/SN38, and OCUM-2M/GEM cells. SN38 decreased the proliferation of OCUM-2M/5FU, OCUM-2M/OXA, and OCUM-2M/GEM. GEM decreased the proliferation of all of chemo-resistant cell lines, except OCUM-2M/GEM (Figure 2).

*Cell death by apoptosis in drug resistant cell lines*. Figure 3 shows the cell death by apoptosis induced by anti-cancer drugs. Anti-cancer drugs were added to the cancer cell cultures at $IC_{50}$ for each cell line. The cell death by apoptosis of anti-cancer drug resistant cell lines, except OCUM-2M/5FU, induced by 5FU, PTX, OXA, SN38, and GEM were lower than half of that of OCUM-2M.

*Cell-cycle distribution*. The percentage of G0/G1 and S phase cells was decreased in OCUM-2M/OXA (13% and 4.5%) and OCUM-2M/SN38 cells (23% and 5%), in comparison with that of control (44% and 46%) (Figure 4).

*Expression of drug resistance related genes detected by RT-PCR*. The expression of *MDR1, MRP, DAPK1, DAPK2, DAPK3, DPD, TK1, TS, TP, MAD2L1, SLC28A3, HMGB1,*

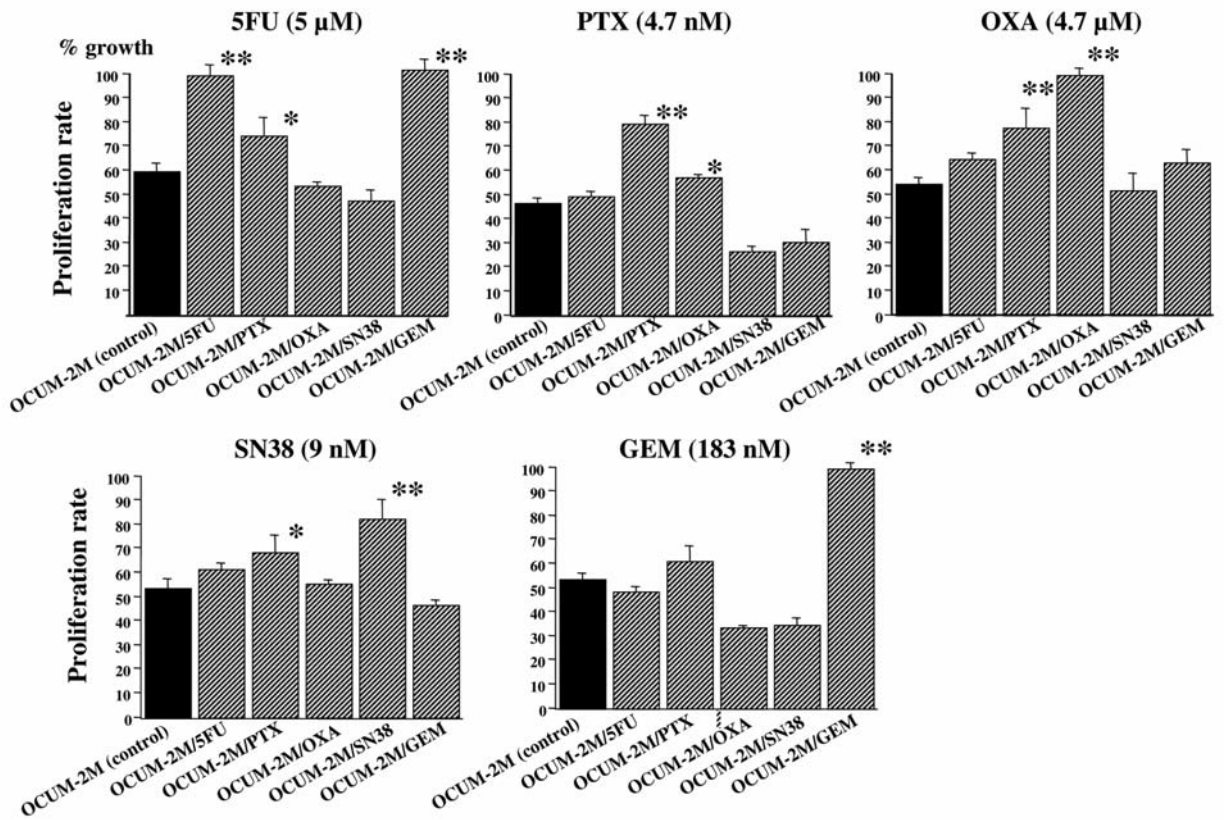


Figure 2. *The effect of chemotherapeutic drugs on the cell proliferation of cancer cells. The proliferation of OCUM-2M/5FU, OCUM-2M/PTX, and OCUM-2M/GEM cells by 5FU exposure was significantly high (99%, 74%, and 101%), compared to that of the parent OCUM-2M cells (59%). The proliferation of OCUM-2M/PTX and OCUM-2M/OXA cells by PTX exposure was significantly high (79% and 57%), compared to OCUM-2M cells (46%). The proliferation of OCUM-2M/PTX and OCUM-2M/OXA cells by OXA exposure was significantly high (77% and 99%), compared to OCUM-2M cells (54%). The proliferation of OCUM-2M/PTX and OCUM-2M/SN38 cells by SN38 exposure was significantly high (68% and 82%), compared to OCUM-2M cells (53%). Only the proliferation of OCUM-2M/GEM cells was significantly high (99%) by GEM exposure. Each cell line was cultured with at the concentration IC50 of parent OCUM-2M cells. Drug sensitivity was determined by an MTT assay. The results are presented as the mean of three independent experiences. *, p<0.05 vs. OCUM-2M (control). **, p<0.01 vs. OCUM-2M (control). 5FU, 5-fluorouracil; PTX, paclitaxel; OXA, oxaliplatin; SN38, irinotecan; GEM, gemcitabine.*

*RRM1, UMPK, TRAG3, Caspase3*, and *P53* was detected in OCUM cells (Figure 5). In the 5 drug resistant cell lines, the expression of *MDR1, MRP* or *DPD* was increased, and the expression of DAPK1 or DAPK2 was decreased. No alteration in the expression of other genes, including *TK1, TS, TP, MAD2L1, SLC28A3, HMGB1, RRM1, UMPK, TRAG3, Caspase3,* and *P53*, was found in any of 5 resistant cell lines.

## Discussion

In this study, 5 anti-cancer drug resistant gastric cancer cell lines were respectively established from the parent gastric cancer cell line, OCUM-2M, by stepwise and continuous exposure to each chemotherapeutical agent. Each resistant cell lines could proliferate under the conditions with the anticancer drug at the concentration from 0.2 to 2 times the $IC_{50}$ of OCUM-2M cells. The RI of the 5 resistant cell lines was greater than 3.0, which suggested that all of resistant gastric cancer cell lines were successfully established to be resistant against 5FU, PTX, OXA, SN38, and GEM, respectively.

RI of OCUM-2M/PTX, OCUM-2M/OXA, and OCUM-2M/GEM cells was over 3.0 in more than 2 drugs, while that of OCUM-2M/5FU or OCUM-2M/SN38 cells was over 3.0 in 5FU or SN38 only. In addition, PTX and OXA were effective for both 5FU resistant cell lines and SN38 resistant cell lines. GEM was effective against all of 4 resistant gastric cancer cell lines. These findings indicated that patients who fail to respond to 5FU and SN38 might still be sensitive to other various anticancer drugs, and indicated that PTX, OXA and GEM resistant cells showed multi-drug resistance. 5FU and SN38 might be appropriate for first-line chemotherapy for gastric cancer, and PTX and OXA might be appropriate for second-line chemotherapy. A GEM-based regimen might be an appropriate second- or third-line chemotherapy for

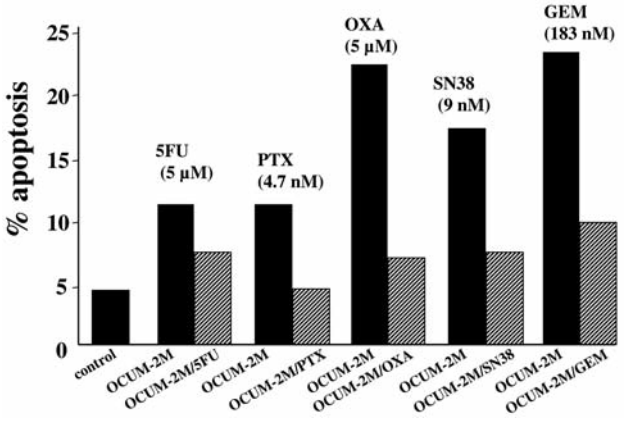


Figure 3. *Apoptotic cell death by anti-cancer drugs. Cells staining annexin V-FITC positive, propidium iodide negative were considered to be apoptotic. In OCUM-2M cells, the apoptotic cell death induced by 5FU, PTX, OXA, SN38, and GEM alone were 11%, 11%, 22%, 17%, and 23% respectively. In contrast, the apoptotic cell death of OCUM-2M/5FU by 5FU, OCUM-2M/PTX by PTX, OCUM-2M/OXA by OXA, OCUM-2M/SN38 by SN38, and OCUM-2M/GEM by GEM were 7.3%, 4.4%, 6.8%, 7.3%, and 9.6%, respectively.*

gastric cancer with multidrug resistance, while GEM is not a clinical agent in gastric cancer so far (13).

The percentage of cells in $G_0/G_1$ phase and S phase was decreased in OCUM-2M/OXA cells and OCUM-2M/SN38, compared with OCUM-2M cells. Sensitivity to cisplatin and SN38 reported to be increased in $G_0/G_1$ and S phase, respectively (14). The alteration of cell-cycle distribution might be associated the chemo-resistance to OXA and SN38.

DPD, an enzyme in 5FU catabolism, converts 5FU to dihydrofluorouracil (15), and the enhanced activity of DPD is known to provide tumor cells with resistance to 5FU (16-18). *DPD* mRNA was increased in the 5FU resistant gastric cancer cell line, OCUM-2M/5FU. In addition, the expression of multidrug resistance related genes, *MDR1* and *MRP*, was increased in OCUM-2M/5FU, OCUM-2M/PTX and OCUM-2M/GEM. All of these cell lines were resistant to 5FU. These findings suggested that MRP, MDR1, and DPD might be associated the chemo-resistance to 5FU. DAPK-1 and 2 are responsible for the induction of apoptosis (19). The *DAPK1* or *DAPK2* expression level was low in OCUM-2M/PTX, OCUM-2M/OXA, and OCUM-2M/SN38. DAPK-1 and 2 might be associated the chemo-resistance to PTX, OXA, or SN38.

Since this study was performed using a parent cancer gastric line OCUM-2M only, the establishment of more multi-drug resistant cell lines is therefore necessary to confirm the above possibility in the future.

In conclusion, 5 cancer cell lines resistant to 5FU, PTX, OXA, SN38 and GEM, were respectively established from the gastric cancer cell line OCUM-2M. *DPD, MDR1 MRP, DAPK1*, and *DAPK2* genes might be associated with the acquisition of anti-cancer drug resistance. PTX, OXA, and

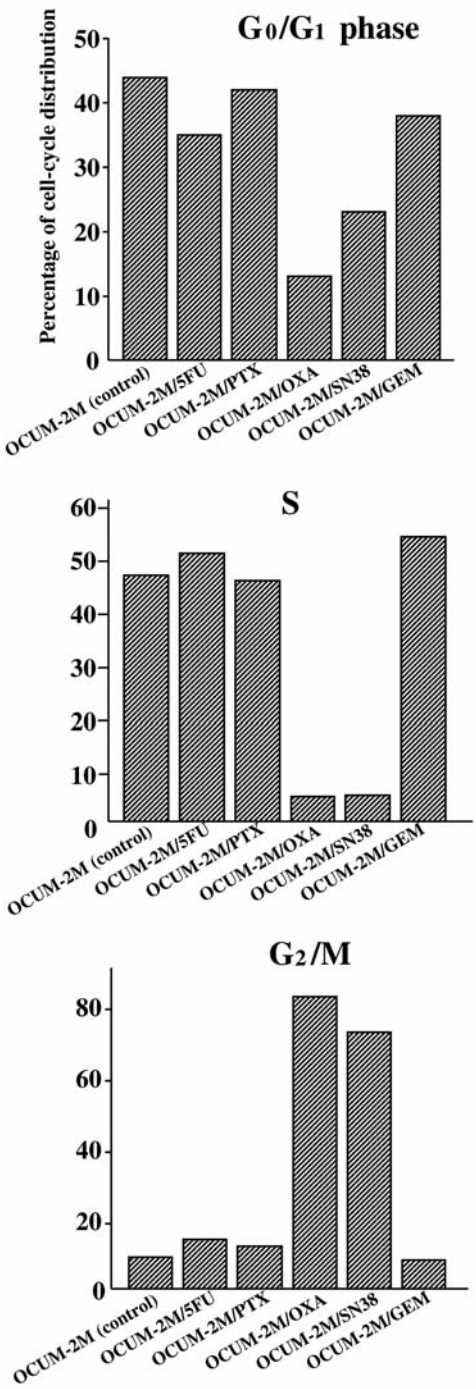


Figure 4. *Cell cycle distribution. In OCUM-2M/OXA and OCUM-2M/SN38 cells, the percentage of $G_0/G_1$ phase and S phase cells was decreased, in comparison with that of OCUM-2M (control) cells. In contrast, no effect of cell cycle distribution was observed in other cell lines.*

GEM resistant cells showed multi-drug resistance, but not 5FU and SN38 resistant cells. These anticancer resistance cell lines may be useful to study the multi-drug resistance in gastric cancer.

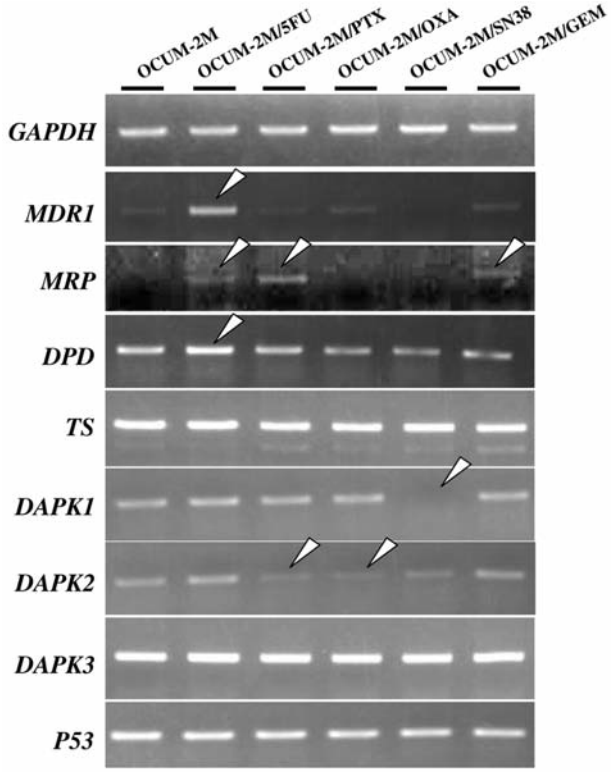


Figure 5. *mRNA expression in drug resistant cell lines. The expression of the genes associated with chemotherapy-resistance was compared among the parent line and 5 drug resistant cell lines. The 5FU resistant cell line, OCUM-2M/5FU, showed increased expression of MDR1, MRP and DPD. OCUM-2M/PTX cells showed the up-regulation of MRP and the down-regulation of DAPK2. OCUM-2M/OXA cells showed the up-regulation of DAPK2. The expression of DAPK1 decreased in the OCUM-2M/SN38 cells. OCUM-2M/GEM cells showed the up-regulation of MRP. The arrowheads indicate the alterations in the gene expression level.*

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