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# Establishment of a Drug Sensitivity Panel Using Human Lung Cancer Cell Lines

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We established a drug sensitivity panel consisting of 24 human lung cancer cell lines. Using this panel, we evaluated 26 anti-cancer agents: three alkylators, three platinum compounds, four antimetabolites, one topoisomerase I inhibitor, five topoisomerase II inhibitors, seven antimitotic agents and three tyrosine kinase inhibitors. This panel showed the following: a) Drug sensitivity patterns reflected their clinically-established patterns of action. For example, doxorubicin and etoposide were shown to be active against small cell lung cancer cell lines and mitomycin-C and 5-fluorouracil were active against non-small cell lung cancer cell lines, in agreement with clinical data. b) Correlation analysis of the mean graphs derived from the logarithm of $IC_{50}$ values of the drugs gave insight into the mechanism of each drug's action. Thus, two drug combinations with reverse or no correlation, such as the combination of cisplatin and vinorelbine, might be good candidates for the ideal two drug combination in the treatment of lung cancer, as is being confirmed in clinical trials. c) Using cluster analysis of the cell lines in the panel with their drug sensitivity patterns, we could classify the cell lines into four groups depending on the drug sensitivity similarity. This classification will be useful to elucidate the cellular mechanism of action and drug resistance. Thus, our drug sensitivity panel will be helpful to explore new drugs or to develop a new combination of anti-cancer agents for the treatment of lung cancer.

**Key words:** drug screening system, MTT assay, lung cancer cell line, drug resistance

Lung cancer is the leading cause of cancer mortality in Japan and in the majority of industrialized countries, and its incidence is still increasing in many developing countries (1, 2). Small cell lung cancer (SCLC) is one of the solid tumors most sensitive to anti-cancer agents, and the objective response rate with recent combination chemotherapy exceeds 80 % even in patients with extensive disease (ED) of SCLC. However, the majority of those patients who respond to such chemotherapy relapse and die within two years (3, 4). In non-small cell lung cancer (NSCLC), standard chemotherapy has not been established for the treatment of patients with advanced NSCLC at stage IV, though the response rate can be improved by using a cisplatin-containing regimen (5).

Recent trials such as non-cross-resistant alternating chemotherapy (6, 7) and dose-intensive chemotherapy (8, 9), which are intended to overcome drug resistance or increase response rates, have produced only marginal improvement in survival. Accordingly, to improve the treatment outcome in advanced lung cancer, the identification and selection of new agents with substantial activities against lung cancer will be needed.

In an attempt to explore new agents active against lung cancer, we tried to establish a panel of human lung cancer cell lines. The primary objectives of this study included: a) the establishment of a panel of human lung cancer cell lines b) the confirmation of its usefulness for evaluation of new cytotoxic agents and c) the evaluation of the optimal two drug combination *in vitro*. The secondary objective was to classify the cell line panel into several groups based on their sensitivity patterns to anti-cancer agents, which will be useful in elucidating the mechanisms of action and resistance anti-cancer agents.

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## Materials and Methods

***Cell lines.*** The cell line panel consisted of 24 human lung cancer cell lines, which included ten small cell carcinoma, eight adenocarcinoma, four squamous cell carcinoma and two large cell carcinoma cell lines. Among these cell lines, SBC-2 (JCRB0817), SBC-3 (JCRB-0818), SBC-4, SBC-5 (JCRB0819), SBC-6, SBC-7, SBC-9B and SBC-10, ABC-1 (JCRB0815), ABC-3, ABC-5 and EBC-1 (JCRB0820) were established in our laboratory (10, 11, 12). Lu-134-A (JCRB0235), PC-3 (JCRB0077), RERF-LC-MS (JCRB0081), LK-2 (JCRB0829), A549 (JCRB0076) and LU99C (JCRB-0058) were provided by the Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan). PC-14 (RCB-0446) and SQ-5 (RCB0110) were purchased from the Riken Cell Bank (RCB, Ibaragi, Japan). NCI-H23 (ATCC CRL5800), NCI-H69 (ATCC HTB-119), NCI-H460 (ATCC HTB-177) and NCI-H520 (ATCC HTB-182) were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA).

All cell lines were maintained in tissue culture flasks in humidified conditions at 37°C in air supplemented with 5 % $CO_2$. The culture medium used in this study was RPMI-1640 (Life Technologies, Inc., Grand Island, NY, USA) supplemented with 10 % fetal bovine serum (ICN Biomedicals Japan Co., Ltd., Tokyo), penicillin-G (100 U/ml) and streptomycin (100 $\mu$g/ml) (RPMI-FBS).

***Chemicals and reagents.*** Twenty-six drugs were tested in this study: cisplatin (CDDP), etoposide (ETP), carboplatin (CBDCA) and paclitaxel (PCT) were provided by Bristol-Myers Squibb K.K., Tokyo; vindesine (VDS), 4-hydroperoxycyclophosphamide (4-HC: an active metabolite of cyclophosphamide), 4-hydroperoxyifosfamide (4-HI: an active metabolite of ifosfamide) and nedaplatin (254-S) by Shionogi & Co., Ltd., Osaka; doxorubicin (DXR), mitomycin-C (MMC), 5-fluorouracil (5-FU) and vinorelbine (VNB) by Kyowa Hakko Kogyo Co., Ltd., Tokyo; vincristine (VCR), vinblastine (VBL) and gemcitabine (dFdC) by Eli Lilly Japan K.K., Kobe, Japan; docetaxel (DCT) by Rhone-Poulenc Rorer, Anton, France; methotrexate (MTX) by Lederle Japan, Ltd., Tokyo; cytarabin (Ara-C: cytosine arabinoside) by Nippon Shinyaku Co., Ltd., Kyoto, Japan; ICRF-154 (an active metabolite of MST-16) by Zenyaku Kogyo Co., Ltd., Tokyo; NK106 and NK611 by Nippon Kayaku Co., Ltd., Tokyo; SN-38 (an active metabolite of irinotecan) by Yakult Honsha Co., Ltd., Tokyo; and rhizoxin (RZX) by Fujisawa Pharmaceutical Co., Ltd., Tokyo. Tyrphostines (AG-370, AG-490 and AG-494) were purchased from BIOMOL Research Laboratories, Inc., Plymouth Meeting, PA, USA.

SN-38, AG-370, AG-490 and ICRF-154 were dissolved in dimethyl sulfoxide (DMSO) and DCT in ethanol. These drug solutions were stored at −20°C and were diluted with tissue culture medium just before use. The other drugs were dissolved and diluted just before use. DXR, VDS, VCR, VBL, dFdC and NK109 were dissolved in isotonic sodium chloride, MTX, 4-HC, 4-HI, MMC and 254-S in distilled water, NK-611 in 5 % glucose solution, RZX in ethanol and AG-494 in DMSO. DMSO, ethanol, isotonic sodium chloride and distilled water in final concentrations did not affect the cell growth (data not shown).

3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Chemical Co., St. Louis, MO, USA.

***Drug sensitivity assay.*** Drug sensitivity was evaluated utilizing the MTT assay (13) with minor modifications (14). Briefly, 50 $\mu$l of RPMI-FBS containing serial concentrations of cytotoxic agents or 50 $\mu$l of RPMI-FBS without agents was plated in a 96-well flat bottomed microplate. Fifty $\mu$l of tumor cell suspension containing 2,000 ~ 20,000 cells from each cell line in RPMI-FBS was plated to each well of the microplate. The plates were incubated at 37°C for 96 h. Then 10 $\mu$l of phosphate-buffered saline containing 50 $\mu$g MTT was added to each well and incubated for 4 h. After the addition of 125 $\mu$l of fresh isopropanol with 0.04 N HCl to each well, microplates were vigorously shaken for 2 min. The absorbance of the wells was measured at 560 nm using a Model 3550 microplate reader (Bio-Rad Laboratories, Richmond, CA, USA). The absorbances of the wells containing drug-free medium with tumor cells and culture medium alone were measured as a control and blank calibration (to 0 absorbance), respectively. The surviving cell fraction was calculated using the following formula:

[(Mean absorbance in four test wells-mean absorbance in blank wells)/(Mean absorbance in four control wells-mean absorbance in blank wells)] × 100.

The concentration of each drug necessary to inhibit the growth of tumor cells by 50 % ($IC_{50}$) was determined by plotting the surviving cell fraction to a drug concentration with a custom-made program in Excel (Microsoft) based

on linear interpolation between data points. Determinations were made from the mean values in quadruplicate. $IC_{50}$ values were compared by two-sided unpaired *t*-test.

***Mean graph patterns.*** Sensitivity data obtained from our human lung cancer cell line panel for anti-cancer agents is indicated as the mean graph developed by the National Cancer Institute (NCI, Bethesda, MD, USA) (NCI mean graph) (15). The mean graph consists of a delta (projecting bar) to the right or left of the mean, depending on whether sensitivity of the cell line to a test drug is more or less than the average. Zero in the graph indicates the mean of the logarithm of $IC_{50}$ values of all cell lines in the panel. For example, a negative value displayed as a delta to the right of the mean, indicates that the cell line is more sensitive than average. In contrast, a positive value, with a delta to the left of the mean, indicates that the cell line is more resistant than average. The length of the delta is proportional to the difference between the logarithm of $IC_{50}$ value for the corresponding cell line and the average.

Pearson's correlation coefficient (r) was employed for comparing the mean graph of a drug with the mean graphs of the remaining drugs.

***Cluster analysis.*** Cluster analysis is a multivariate analytical method used to recognize complex information as patterns. Clustering means the grouping of similar objects using algorithms to reduce and simplify the information. We applied this analysis to classify the lung cancer cell lines used in our cell line panel by their sensitivities to 9 anti-cancer agents. In this analysis, instead of the former "NCI mean graph", we employed the drug sensitivity graph to characterize the cell lines depending on their sensitivity patterns to anti-cancer agents. For a given cell line, this graph illustrates the delta (the projecting bar of the NCI mean graph) to the representative 9 anti-cancer agents which were arbitrarily selected from anti-cancer agents frequently used in the treatment of lung cancer and from new drugs.

We used a JMP (Version 3.01, SAS institute Inc., Cary, NC, USA) statistical software package for calculating Pearson's correlation coefficient and for the cluster analysis according to Ward's method (16).

## Results

***Cytotoxicity of drugs against the drug sensitivity panel.*** Table 1 shows the summarized data of mean $IC_{50}$ values of the drugs for SCLC and NSCLC cell lines. 5-FU, SN-38, ETP and NK611 showed large standard deviations, indicating that these drugs showed a wide range of $IC_{50}$ values to the lung cancer cell lines. $IC_{50}$ values to 5-FU ranged from 656 nM (PC-14) to 110,000 nM (NCI H69). Similarly, $IC_{50}$ values to SN-38 ranged from 0.296 nM (SBC-6) to 271 nM (NCI-H520). $IC_{50}$ values to ETP and NK611 ranged from 247 nM and 1,670 nM (SBC-3) to 122,000 nM and 203,000 nM (SBC-4), respectively. In contrast, $IC_{50}$ values to 4-HC, 4-HI, ICRF-154, DCT, PCT, VBL, AG-494, AG-370 and AG-490 showed small ranges. Of note, $IC_{50}$ values of DCT and PCT only ranged from 0.580 nM and 1.07 nM (SBC-3) to 5.30 nM and 16.4 nM (Lu-134-A), respectively.

NSCLC cell lines were more than twice as sensitive as SCLC cell lines to MMC, 5-FU and Ara-C in terms of mean $IC_{50}$ values. SCLC cell lines were more than twice as sensitive as compared with NSCLC cell lines to 4-HC, 4-HI, SN-38 and VCR.

***Comparison of mean graph pattern.*** Fig. 1 shows the NCI mean graph profiles for platinum compounds (CDDP, CBDCA and 254-S, which possess the same carrier ligand with different leaving sites) and the antimitotic agent, VNB. VNB showed a different pattern of cytotoxic activity from the platinum compounds. CDDP showed a significant correlation with CBDCA ($r = 0.879$, $P < 0.0001$) and 254-S ($r = 0.880$, $P < 0.0001$) respectively, whereas CDDP was not correlated with VNB ($r = 0.0205$) (Fig. 2). In this study, 4-HC, DXR, CDDP, ETP and VDS as standard lung cancer drugs, dFdC, PCT, SN-38 and VNB as new active drugs for lung cancer, and AG-490 as a test drug were selected to search for new drugs with different mechanisms of activity against lung cancer. The above-mentioned drugs were used as reference drugs and the correlation coefficients of all drugs used in this study are shown in Tables 2-1 and 2-2.

Platinum compounds (254-S, CBDCA) showed the highest correlation coefficients with CDDP, while VDS, RZX (antimitotic agent) and AG-370 (tyrosine kinase inhibitor) showed the lowest. Similarly, drug combinations showing good correlation included analogues such as VDS *vs* VCR ($r = 0.925$, $P < 0.0001$), VNB ($r = 0.916$, $P < 0.0001$) and VBL ($r = 0.786$, $P < 0.0001$), VNB *vs* VBL ($r = 0.903$, $P < 0.0001$) and VCR ($r = 0.814$, $P < 0.0001$), 4-HC *vs* 4-HI ($r = 0.862$, $P < 0.0001$), and PCT *vs* DCT ($r = 0.459$, $P = 0.024$), but not Ara-C *vs* dFdC ($r = 0.411$, $P = 0.0572$). Roughly,

**Table I** Mean $IC_{50}$ values in small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) cell lines (nM)

| | | SCLC | | NSCLC | | All | |
|---|---|---|---|---|---|---|---|
| | | m | ± S.D. | m | ± S.D. | m | ± S.D. |
| Alkylator | MMC | 411 | 709 | 186 | 175 | 280 | 476 |
| | 4-HC | 2780 | 1650** | 6100 | 3620** | 4720 | 3360 |
| | 4-HI | 9540 | 4410* | 19000 | 11500* | 15100 | 10200 |
| Platinum compound | CDDP | 4870 | 7550 | 4390 | 5110 | 4590 | 6090 |
| | 254-S | 7100 | 13100 | 7410 | 9520 | 37500 | 10900 |
| | CBDCA | 36900 | 67800 | 37900 | 31800 | 37500 | 48700 |
| Antimetabolite | dFdC | 6.87 | 7.86 | 8.29 | 10.4 | 7.70 | 9.25 |
| | MTX | 59.4 | 72.4 | 63.4 | 75.4 | 61.8 | 72.5 |
| | Ara-C | 1300 | 1810 | 453 | 392 | 799 | 1230 |
| | 5-FU | 18700 | 33200 | 5000 | 4370 | 11000 | 22600 |
| Topoisomerase I inhibitor | SN-38 | 6.38 | 13.4 | 30.0 | 70.7 | 20.1 | 55.1 |
| Topoisomerase II inhibitor | DXR | 136 | 156 | 227 | 231 | 189 | 204 |
| | NK109 | 518 | 506 | 619 | 677 | 577 | 601 |
| | ETP | 14800 | 38100 | 10400 | 24600 | 12200 | 30300 |
| | NK611 | 29700 | 61600 | 15100 | 22300 | 21200 | 42700 |
| | ICRF | 197000 | 63800 | 253000 | 75800 | 230000 | 74900 |
| Antimitotic agent | DCT | 2.38 | 1.76 | 1.83 | 0.657 | 2.06 | 1.24 |
| | VBL | 4.25 | 4.64 | 4.45 | 1.69 | 4.37 | 3.17 |
| | PCT | 4.66 | 4.30 | 5.36 | 3.14 | 5.07 | 3.60 |
| | RZX | 5.07 | 3.58 | 5.69 | 5.02 | 5.44 | 4.44 |
| | VDS | 10.5 | 26.4 | 8.68 | 7.48 | 9.46 | 17.4 |
| | VNB | 11.1 | 18.0 | 10.5 | 5.73 | 10.8 | 12.0 |
| | VCR | 7.51 | 16.4 | 15.0 | 26.8 | 12.1 | 23.1 |
| Tyrosine kinase inhibitor | AG-494 | 20900 | 12500 | 28300 | 11300 | 25100 | 12200 |
| | AG-370 | 30600 | 8050 | 26400 | 6720 | 28100 | 7440 |
| | AG-490 | 32900 | 13000 | 32200 | 6320 | 32500 | 9400 |

m: Mean; S.D.: Standard deviation; ICRF: ICRF-154; $^{**}P = 0.013$; $^{*}P = 0.022$.

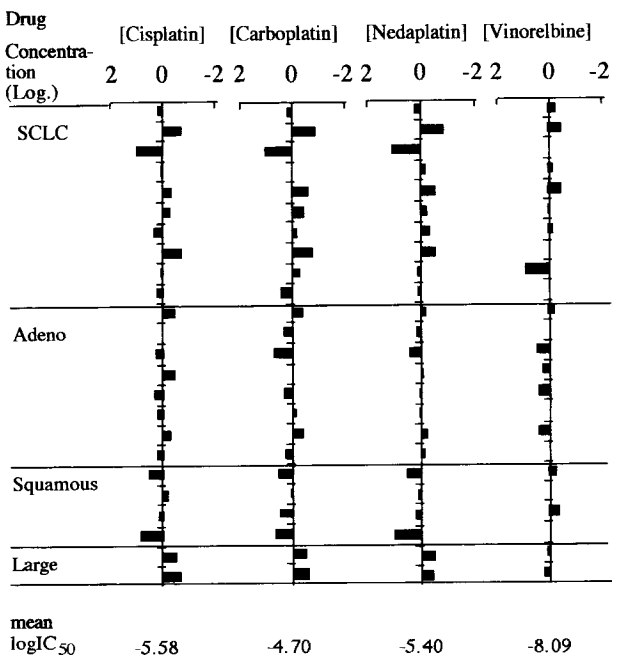


**Fig. 1** Comparison of mean graph patterns of cisplatin with those of carboplatin, nedaplatin, and vinorelbine. The cell lines are (listed from top to bottom): small cell lung cancer (SCLC) — SBC-2, SBC-3. SBC-4, SBC-5, SBC-6, SBC-7, SBC-9B, SBC-10, Lu-134-A and NCI-H69; adenocarcinoma — ABC-1, ABC-3, ABC-5, A549, PC-3, PC-14, RERF-LC-MS and NCI-H23; squamous cell carcinoma — EBC-1, SQ-5, LK-2 and NCI-H520 and; large cell carcinoma — LU99C and NCI-H460.

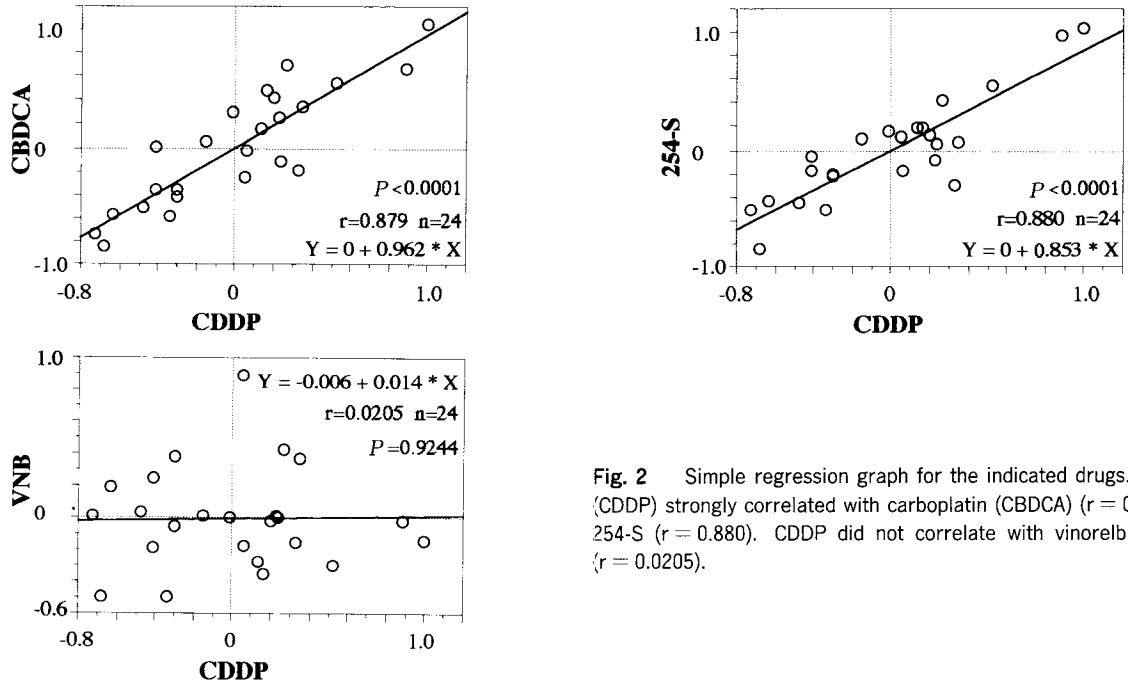


**Fig. 2** Simple regression graph for the indicated drugs. Cisplatin (CDDP) strongly correlated with carboplatin (CBDCA) (r = 0.879) and 254-S (r = 0.880). CDDP did not correlate with vinorelbine (VNB) (r = 0.0205).

**Table 2-1** Rank list of the correlation coefficients (r) among all drugs tested using 4-HC, DXR, CDDP, ETP and VDS as reference drugs

| | 4-HC | | DXR | | CDDP | | ETP | | VDS |
|---|---|---|---|---|---|---|---|---|---|
| 4-HI | 0.862** | NK109 | 0.875** | 254-S | 0.880** | 254-S | 0.812** | VCR | 0.925** |
| SN-38 | 0.728** | 254-S | 0.818** | CBDCA | 0.879** | CDDP | 0.760** | VNB | 0.916** |
| VCR | 0.618** | CBDCA | 0.813** | ETP | 0.760** | NK611 | 0.755** | VBL | 0.786** |
| DXR | 0.584** | NK611 | 0.798** | NK611 | 0.734** | DXR | 0.736** | PCT | 0.612** |
| CBDCA | 0.545** | ETP | 0.736** | MMC | 0.714** | CBDCA | 0.726** | 4-HC | 0.430** |
| NK109 | 0.513* | SN-38 | 0.720** | DXR | 0.701** | dFdC | 0.684** | 4-HI | 0.420* |
| 254-S | 0.505* | CDDP | 0.701** | MTX | 0.591** | SN-38 | 0.679** | RZX | 0.293 |
| ETP | 0.448* | 4-HI | 0.665** | Ara-C | 0.589** | MMC | 0.662** | Ara-C | 0.291 |
| VDS | 0.430* | MMC | 0.650** | SN-38 | 0.575** | NK109 | 0.652** | DCT | 0.287 |
| AG-494 | 0.422* | dFdC | 0.592** | NK109 | 0.570** | AG-494 | 0.517* | AG-494 | 0.277 |
| dFdC | 0.401 | 4-HC | 0.584** | 4-HI | 0.567** | 4-HI | 0.478* | AG-490 | 0.217 |
| MMC | 0.395 | PCT | 0.546** | dFdC | 0.475* | Ara-C | 0.451* | MTX | 0.191 |
| VBL | 0.395 | MTX | 0.545** | AG-494 | 0.459* | 4-HC | 0.448* | DXR | 0.162 |
| VNB | 0.393 | AG-494 | 0.479* | DCT | 0.374 | DCT | 0.412* | 254-S | 0.145 |
| CDDP | 0.372 | ICRF | 0.477* | 4-HC | 0.372 | MTX | 0.365 | dFdC | 0.143 |
| NK611 | 0.326 | DCT | 0.358 | PCT | 0.344 | ICRF | 0.328 | 5-FU | 0.109 |
| PCT | 0.318 | Ara-C | 0.316 | ICRF | 0.209 | PCT | 0.301 | NK109 | 0.101 |
| Ara-C | 0.293 | VBL | 0.300 | AG-490 | 0.203 | VBL | 0.271 | SN-38 | 0.0983 |
| ICRF | 0.140 | VCR | 0.268 | VBL | 0.171 | AG-490 | 0.211 | CBDCA | 0.0877 |
| AG-490 | 0.113 | 5-FU | 0.262 | VCR | 0.122 | 5-FU | 0.183 | ICRF | −0.0111 |
| RZX | 0.100 | VNB | 0.223 | VNB | 0.0719 | VCR | 0.127 | CDDP | −0.0130 |
| 5-FU | −0.0121 | AG-490 | 0.212 | 5-FU | 0.0547 | VNB | 0.0889 | ETP | −0.0148 |
| MTX | −0.0135 | VDS | 0.162 | VDS | −0.0130 | VDS | −0.0148 | MMC | −0.0392 |
| DCT | −0.0268 | RZX | 0.0103 | RZX | −0.0843 | AG-370 | −0.0199 | NK611 | −0.0788 |
| AG-370 | −0.0211 | AG-370 | −0.0342 | AG-370 | −0.134 | RZX | −0.159 | AG-370 | −0.188 |

ICRF: ICRF-154; An active metabolite of MST-16; $^{**}P < 0.01$; $^{*}P < 0.05$. 4-HC: An active metabolite of cyclophosphamide; DXR: Doxorubicin; CDDP: Cisplatin; ETP: Etoposide; VDS: Vindesine; 4-HI: An active metabolite of ifosfamide; SN-38: An active metabolite of irinotecan; VCR: Vincristine; CBDCA: Carboplatin; 254-S: Nedaplatin; dFdC: Gemcitabine; MMC: Mitomycin-C; VBL: Vinblastine; VNB: Vinorelbine; PCT: Paclitaxel; Ara-C: Cytarabin; RZX: Rhizoxin; 5-FU: 5-fluoronracil; MTX: Methotrexate; DCT: Docetaxel.

**Table 2-2** Rank list of the correlation coefficients (r) among all drugs tested using AG-490, dFdC, PCT, SN-38 and VNB as reference drugs

| | AG-490 | | dFdC | | PCT | | SN-38 | | VNB |
|---|---|---|---|---|---|---|---|---|---|
| AG-494 | 0.626** | ETP | 0.684** | VNB | 0.624** | 4-HI | 0.769** | VDS | 0.916** |
| AG-370 | 0.478* | SN-38 | 0.660** | VDS | 0.612** | 4-HC | 0.728** | VBL | 0.903** |
| PCT | 0.439* | 254-S | 0.605** | NK109 | 0.558** | DXR | 0.720** | VCR | 0.814** |
| MTX | 0.373 | DXR | 0.592** | DXR | 0.546** | ETP | 0.679** | PCT | 0.624** |
| dFdC | 0.359 | AG-494 | 0.586** | VCR | 0.524* | CBDCA | 0.664** | 4-HI | 0.400 |
| NK611 | 0.354 | NK611 | 0.582** | MTX | 0.522* | dFdC | 0.660** | RZX | 0.397 |
| ICRF | 0.316 | DCT | 0.519** | 254-S | 0.480* | 254-S | 0.655** | 4-HC | 0.393 |
| NK109 | 0.306 | NK109 | 0.516** | VBL | 0.476* | NK109 | 0.615** | DCT | 0.388 |
| DCT | 0.294 | CDDP | 0.475* | DCT | 0.459* | NK611 | 0.611** | Ara-C | 0.312 |
| VCR | 0.229 | MMC | 0.466* | AG-490 | 0.439* | CDDP | 0.575** | dFdC | 0.274 |
| VBL | 0.224 | CBDCA | 0.461* | 4-HI | 0.397 | MMC | 0.551** | DXR | 0.223 |
| VDS | 0.217 | 4-HI | 0.460* | CBDCA | 0.345 | AG-494 | 0.491* | 254-S | 0.219 |
| DXR | 0.212 | MTX | 0.452* | CDDP | 0.344 | ICRF | 0.406* | AG-490 | 0.191 |
| ETP | 0.211 | VBL | 0.434* | Ara-C | 0.336 | VCR | 0.353 | AG-494 | 0.177 |
| CDDP | 0.203 | Ara-C | 0.411 | ICRF | 0.335 | PCT | 0.280 | MMC | 0.145 |
| VNB | 0.191 | 4-HC | 0.401 | dFdC | 0.325 | VBL | 0.270 | 5-FU | 0.144 |
| 254-S | 0.157 | AG-490 | 0.359 | 4-HC | 0.318 | MTX | 0.216 | NK109 | 0.135 |
| Ara-C | 0.154 | ICRF | 0.357 | ETP | 0.301 | Ara-C | 0.140 | MTX | 0.133 |
| SN-38 | 0.137 | PCT | 0.325 | NK611 | 0.296 | AG-495 | 0.137 | SN-38 | 0.125 |
| 4-HC | 0.113 | VCR | 0.295 | SN-38 | 0.280 | VNB | 0.125 | CBDCA | 0.117 |
| 4-HI | 0.0431 | VNB | 0.274 | AG-494 | 0.272 | DCT | 0.115 | ETP | 0.089 |
| CBDCA | 0.0425 | 5-FU | 0.182 | 5-FU | 0.235 | VDS | 0.098 | CDDP | 0.072 |
| RZX | 0.0175 | VDS | 0.143 | AG-370 | 0.215 | AG-370 | −0.150 | NK611 | 0.044 |
| MMC | 0.0168 | RZX | 0.0255 | MMC | 0.189 | RZX | −0.176 | ICRF | −0.00420 |
| 5-FU | −0.0473 | AG-370 | −0.129 | RZX | −0.0227 | 5-FU | −0.253 | AG-370 | −0.106 |

ICRF: ICRF-154; $^{**}P < 0.01$; $^{*}P < 0.05$.

this panel identified the drugs with similar action mechanisms, consistent with Paull's data (15).

DXR was highly correlated with topoisomerase I & II inhibitors, platinum compounds and alkylators. ETP was highly correlated with platinum compounds, topoisomerase I & II inhibitors and alkylators. PCT was not correlated with 4-HI, CDDP, CBDCA, dFdC, 4-HC, ETP, SN-38, 5-FU and MMC. VNB was significantly correlated with vinca alkaloids and PCT, but was not with the remaining drugs tested. dFdC was not correlated with Ara-C, 4-HC, PCT, VCR, VNB, 5-FU, VDS and RZX. SN-38 was significantly correlated with alkylators, platinum compounds, topoisomerase II inhibitors and dFdC, but was not with 5-FU, RZX, vinca alkaloids, taxanes, Ara-C and MTX.

AG-490, AG-494 and AG-370 inhibit tyrosine kinase, and probably possess the same mechanism of drugs activity. AG-490 was correlated with AG-494 ($r = 0.626$, $P = 0.014$) and AG-370 ($r = 0.478$, $P = 0.0188$). However, AG-490 was not correlated with any other drugs tested except for PCT. Consequently, this panel demonstrated that AG-490 possessed the same mechanism of drug activity as AG-494 and AG-370, and this mechanism was different than those of the other drugs tested.

RZX was not correlated with any other drugs tested. The highest correlation coefficient value with RZX was VNB ($r = 0.397$, $P = 0.0607$).

***Clustering of cell lines.*** Based on these sensitivity patterns obtained by cluster analysis, the cell lines in the panel could be divided into four groups. Fig. 3 shows result of the cluster analysis. Using algorithms, a variance matrix between the clusters (cells) was calculated, and two clusters with the least variance were joined into one cluster. For example, the variance between SBC-7 and ABC-3 was least among all matrixes between the clusters, so they were joined into one cluster first. Likewise, all of the clusters were joined. Using this cluster analysis, 4 representative cells of the clustering cell lines were selected. LK-2 with the fewest branches in the cluster tree was selected as a representative cell line

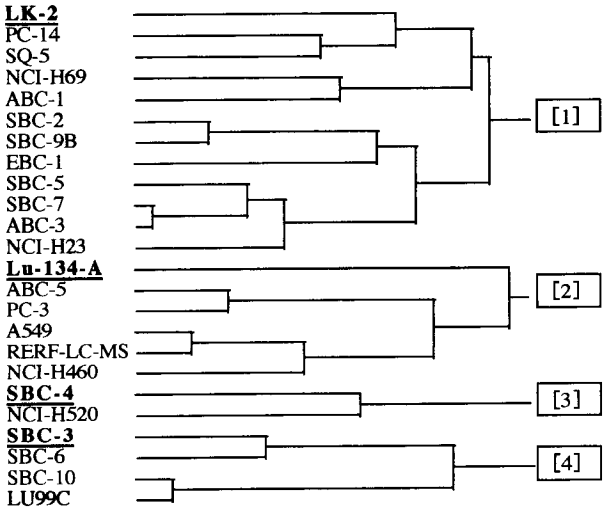


**Fig. 3** Cluster analysis.

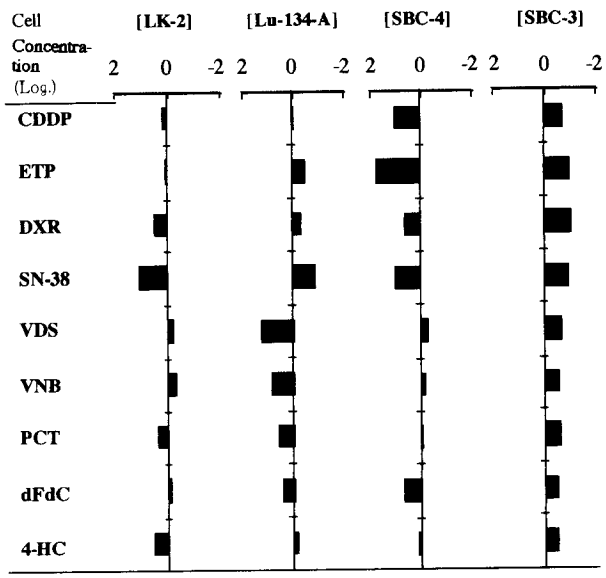


**Fig. 4** Drug sensitivity graph. Each pattern of the 4 representative cell lines is markedly different.

out of PC-14, SQ-5, NCI-H69, ABC-1, SBC-2, SBC-9B, EBC-1, SBC-5, SBC-7, ABC-3 and NCI-H23. Similarly, Lu-134-A, SBC-4 and SBC-3 were selected as representative cell lines out of the clustering cell lines.

Sensitivities of the representative cell lines, LK-2, Lu-134-A, SBC-4 and SBC-3 for the drugs commonly used in the treatment of lung cancer and for the new drugs were displayed (Fig. 4), and converted into a spider graph

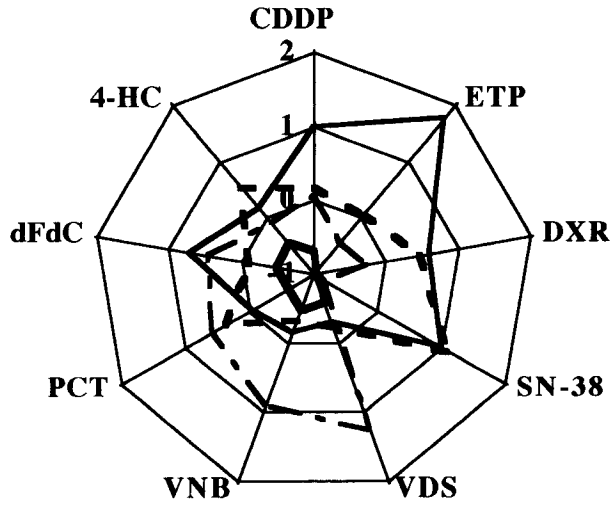


**Fig. 5** Spider graph. In each group divided by cluster analysis (Fig. 3), a representative cell line was chosen (LK-2 for group [1], Lu-134-A for group [2], SBC-4 for group [3] and SBC-3 for group [4]).
▬ ▬: LK-2; ▬ ▬ ▬: Lu-134-A; ▬▬▬: SBC-4; ▬▬▬: SBC-3.

(Fig. 5). LK-2 in group 1 was a moderately resistant cell line but sensitive to vinca alkaloids. Lu-134-A in group 2 was a moderately sensitive cell line but resistant to tublin inhibitors and dFdC. LK-2 and Lu-134-A showed almost diametrically-opposed sensitivity. On the other hand, SBC-4 in group 3 was the most resistant cell line and slightly sensitive to tublin inhibitors. Finally, SBC-3 in group 4 was the most sensitive cell line to all drugs.

## Discussion

In an attempt to identify potentially active new anticancer agents in a disease-oriented manner, the NCI has established an automated *in vitro* drug screening system that uses 60 different human tumor cell lines derived from nine types of cancer (central nervous system, colon, renal, leukemia, lung, melanoma, ovarian, prostate and breast). This system utilizes the mean graph pattern to evaluate the sensitivity of a test drug and employs the COMPARE computer program to compare the mean graph pattern of the drug with patterns of other drugs. However, it uses only nine NSCLC cell lines and does not use SCLC cell lines. In the present study, we developed an organ (lung cancer) specific cell line panel consisting of 24 human lung cancer cell lines. MMC and 5-FU

were more effective against NSCLC cell lines than against SCLC cell lines, VCR and 4-HC against SCLC cell lines than against NSCLC cell lines *in vitro*. These results reflect the response rates to these drugs established in clinical practice (17, 18). MMC was demonstrated to be inactive against SCLC cell lines. VCR was active against SCLC cell lines, but not against NSCLC cell lines. We could select certain drugs active against SCLC or NSCLC cell lines using this panel. Platinum compounds and antimitotic agents, active against both SCLC and NSCLC cell lines in a clinical use (17, 18), showed almost equal mean $IC_{50}$ values for both cell lines. Among drugs recently developed and proven to be highly active against lung cancer, dFdC, SN-38, VNB, DCT and PCT (19) showed low mean $IC_{50}$ values. Aside from adverse reactions, this lung cancer specific cell line panel allows one to select candidate drugs for treatment of lung cancer.

AG-490 as an example drug was correlated with AG-494 and AG-370, but not with any other drugs tested except PCT. In general, analogues such as platinum compounds with the same carrier ligand (CDDP, CBDCA, 254-S), vinca alkaloids (VCR, VDS, VBL, VNB), and alkylators (4-HC, 4-HI) showed good correlation. Consequently, this panel demonstrated the detection of a group of drugs sharing the same mechanism or the other new mechanisms. This analysis will make it possible to sort out the mechanism of new drugs or the previously unknown mechanism of the well-known drugs. Of note, SN-38 showed very good correlation with 4-HC and 4-HI. The same mechanism between SN-38 and 4-HC/4-HI is postulated. RZX showed no correlation with any drugs tested. Although RZX is a mitotic inhibitor like vincristine, RZX possesses a different binding site to tubulin from vincristine (20). In addition, VCR-resistant cells retain the sensitivity to RZX (21). Therefore, it is suggested that RZX still has an unknown mechanism of action. Correlation between drugs in this study may provide the means of discovering a new mechanism.

According to the Goldie-Coldman hypothesis, which suggests that drug-resistant cells arise from mutations, inherit and propagate themselves, alternating administration of non cross-resistant drugs is effective against prevent the emergence of drug resistance (22). Two drugs showing good correlation indicate a similar spectrum for lung cancer cell lines; both drugs are effective against the one cell line, not against the other. This indicates that a two-drug combination with good correlation will not eradicate the resistant cancer cells. Correlation analysis of the mean graphs may be useful in selection of non-cross-resistant drugs. Accordingly, one will be able to plan a new combination chemotherapy for lung cancer using this analysis. In fact, CDDP and VNB were effective against lung cancer cell lines *in vitro* and showed no correlation in this panel. Recently, the combination of CDDP and VNB has produced a good response rate and survival in advanced NSCLC (23). Although our *in vitro* drug sensitivity panel appears to reflect the clinical diversity of human lung cancer, the reliability of the prediction of *in vivo* drug sensitivity is a critical problem (24). When these results shown here are confirmed in clinical trials, the usefulness of our drug sensitivity panel will be confirmed.

With cluster analysis, the cell lines in our cell line panel were appropriately classified into 4 groups according to their sensitivity patterns. The 4 representative cell lines in each group selected by cluster analysis may be enough to perform the preclinical evaluation and preliminary classification of anti-cancer agents. Recently, several cellular mechanisms affecting drug resistance (25) have been proposed. These mechanisms include overexpression of P-glycoprotein and multidrug resistance-associated proteins, increase of glutathione-dependent detoxification and alteration of nuclear target enzymes. Grouping of the cell lines with similar sensitivity will enable one to better understand the major cellular mechanism of action and drug resistance. It is necessary to develop a database of these possible molecular targets or modulators affecting drug sensitivity in this lung cancer cell line panel.

In conclusion, we developed a lung cancer-specific human cell line panel. Our data indicate that *in vitro* drug screening for lung cancers using this panel will be informative. Correlation analysis of the mean graph pattern will be useful for selecting non cross-resistant drugs to design a new combination chemotherapy. Classification of the cell lines by the sensitivity pattern to anti-cancer agents using cluster analysis will be also useful to elucidate the cellular mechanism of action and drug resistance. Furthermore, our drug sensitivity panel will be helpful to explore new drugs or to develop new combinations of anti-cancer agents for the treatment of lung cancer.

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