Cancer Chemother Pharmacol (2003) 52 (Suppl 1): S74–S79
DOI 10.1007/s00280-003-0649-1

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Panel of human cancer cell lines provides valuable database for drug discovery and bioinformatics

Published online: 18 June 2003

Abstract Studies conducted at the US National Cancer Institute (NCI) and in our laboratory show that databases including the drug sensitivities of panels of many human cancer cell lines provide valuable information on the molecular pharmacology of anticancer drugs. We established a panel of 39 cell lines of various human cancers and developed a database of their chemosensitivities. Drugs were profiled in terms of their "fingerprints", patterns of differential activity against the cell lines. There was a significant correlation between a drug's fingerprint and its mode of action, as observed in the NCI panel of 60 cell lines. Therefore our cell-line panel is a powerful tool to predict the modes of action of new compounds. We have been using this system for drug discovery, coupled with various target-based drug screenings. We used the system to identify a novel DNA minor-groove binder, MS-247, which has inhibitory activity against topoisomerases I and II, and potent in vivo antitumor activity against various human cancer xenografts. We also discovered a potent novel telomerase inhibitor, FJ5002, by mining our database with the COMPARE algorithm, followed by experimental validation. We investigated the gene expression profiles of the cell lines by using DNA microarrays to find profiles determining cellular chemosensitivity and new targets for anticancer drugs. Our integrated database, including the chemosensitivities and gene expression profiles of the cell-line panel, could provide a basis for drug discovery and personalized therapy.

This work was presented at the 18th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "New Strategies for Novel Anticancer Drug Development", 8–9 November 2002, Nagoya, Japan.

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Keywords Cancer cell-line panel · Anticancer drug discovery · Bioinformatics · Gene expression · Chemosensitivity

Introduction

Developing more effective new agents is essential for the progress of cancer chemotherapy. Recent trends in drug discovery have been toward inhibitors of target molecules for which a causal relationship with cancer malignancy has been demonstrated. Inhibitors of various protein kinases are under development and some have become new chemotherapeutic agents [12]. In addition to known targets, new targets related to the cell cycle, apoptosis, invasion, and angiogenesis are being investigated.

The Screening Committee of New Anticancer Agents is supported by grant-in-aid for scientific research on priority areas from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The committee has nine screens oriented toward different types of targets or mechanisms: a panel of 39 human cancer cell lines, tubulin, topoisomerase I and II, histone deacetylase I, protein kinases, *p*-glycoprotein, invasion, angiogenesis, and apoptosis. We use these to carry out anticancer drug screening.

The panel of 39 human cancer cell lines can predict modes of action, as described below, and plays a central role in the screening. Combining the panel screen with other methods has enabled us to evaluate test compounds efficiently in multiple pharmacological aspects. Here, I report the potential of our panel of cell lines and database of their chemosensitivities to play an important role in drug discovery.

Development of cell lines for anticancer drug screening

A panel of 60 human cancer cell lines for anticancer drug screening was originally established at the US

National Cancer Institute (NCI) [1]. This was initially called a "disease-oriented screen". Paull and colleagues established the COMPARE computer algorithm and showed that the "fingerprints" (growth inhibitory patterns against diverse cancer cell lines) of anticancer drugs correlated well with their modes of action [9]. Based on this finding, the 60 cell-line panel became a unique and useful tool for drug evaluation, which began an information-intensive approach to the study of the molecular pharmacology of cancer [13]. In addition, the potential of the panel has been expanded to research on the relation between chemosensitivity and gene expression profile in cancer cells [10, 11].

We established and are investigating the potential of a panel of 39 human cancer cell lines [15]. Our panel has proved to be a powerful tool in drug evaluation and drug discovery. Its application to postgenomic research has just begun.

Database of chemosensitivities of 39 cell lines

We established a panel of human cancer cell lines combined with a database of drug sensitivities. The system was developed according to the NCI method [1, 7, 9] with modifications [15]. The panel consists of 39 cell lines including various types of cancer (Table 1). We employ six stomach cancer cell lines in view of the high incidence of this cancer in Japan. Three breast cancer cell lines (HBC-4, HBC-5, and BSY-1) established at our institute are used. Other cell lines are also included in the NCI 60 cell-line panel.

Using this system, we tested the antiproliferative effects of more than 300 standard compounds including various anticancer drugs and inhibitors of biological pathways. The details of how cell growth inhibition was measured are described elsewhere [7, 14]. For each drug, a fingerprint—its differential growth inhibitory activity against the cell lines—was created based on a calculation using data for GI_{50}, the concentration required for 50% growth inhibition [1, 9]. To analyze the correlation between the fingerprints of drug A and drug B, the COMPARE algorithm was developed according to the method described by Paull and coworkers [1, 9]. Pearson correlation coefficients were calculated using the formula $r = [\Sigma(x_i - x_m)(y_i - y_m)]/[\Sigma(x_i - x_m)^2 \Sigma(y_i - y_m)^2]^{1/2}$, where x_i and y_i are log GI_{50} of drug A and drug B, respectively, against each cell line, and x_m and y_m are the mean values of x_i and y_i, respectively.

Table 1 Panel of 39 human cancer cell lines used by the Screening Committee of New Anticancer Agents

Cancer	No. of cell lines	Cell lines
Lung	7	NCI-H23, NCI-H226, NCI-H522, NCI-H460, A549, DMS273, DMS114
Stomach	6	St-4, MKN-1, MKN-7, MKN-28, MKN-45, MKN-74
Ovarian	5	OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3
Renal	2	LOX-IMVI, ACHN
Melanoma	1	RXF-631L
Colon	5	HCC-2998, KM-12, HT-29, HCT-15, HCT-116
Breast	5	HBC-4, BSY-1, HBC-5, MCF-7, MDA-MB-231
Brain	6	U251, SF-268, SF-295, SF-539, SNB-75, SNB-78
Prostate	2	DU-145, PC-3

Fingerprint classification of anticancer drugs

Each drug has its own fingerprint. Using the COMPARE algorithm, we compared anticancer drugs based on their fingerprints. This showed that the fingerprint of a drug correlates well with its mode of action, as seen in the NCI 60 cell-line panel [1, 9]. This was more clearly demonstrated by the hierarchical cluster analysis applied to the GI_{50} data of 53 anticancer drugs (Fig. 1). Drugs that share modes of action or targets tend to form clusters. For example, tubulin binders (vincristine, vinblastine, colchicine, dolastatin 10, paclitaxel, docetaxel, E7010, and vinorelbine) and topoisomerase I inhibitors (topotecan, SN-38, and camptothecin) form separate clusters. Oxaliplatin lacks cross-resistance with cisplatin and carboplatin [4] and is located away from the cluster of cisplatin and carboplatin.

We have recently established a different panel of 45 cell lines including stomach, liver, and breast cancers, and again observed good correlation between a drug's fingerprint and its mode of action (data not shown). Therefore it is suggested that such correlation appears in any panel that includes an assortment of cell lines with diverse chemosensitivities.

Potential of the 39 cell-line panel in drug discovery

The application of the cell-line panel database in drug discovery is twofold. First, it can predict the mode of action of a new compound by comparing its fingerprint with those of standard drugs and inhibitors in the database. For example, a synthetic compound, MS-247, with a netropsin-like moiety and an alkylating residue in its structure (Fig. 2) was evaluated [15]. The average GI_{50} against the 39 cell-line panel was 0.71 μM. COMPARE analysis revealed that MS-247 significantly correlated with camptothecin analogs (topoisomerase I inhibitors) and anthracyclines (topoisomerase II inhibitors), suggesting that MS-247 shares some modes of action with these two drug groups. Investigation of the mode of action of MS-247 showed that it induced G_2/M arrest and apoptosis in cancer cells and, more precisely, showed DNA-binding activity and inhibited topoisomerases I and II, as expected from the COMPARE analysis. Moreover, MS-247 exhibited potent antitumor activity when administered as a single intravenous injection against various xenografts

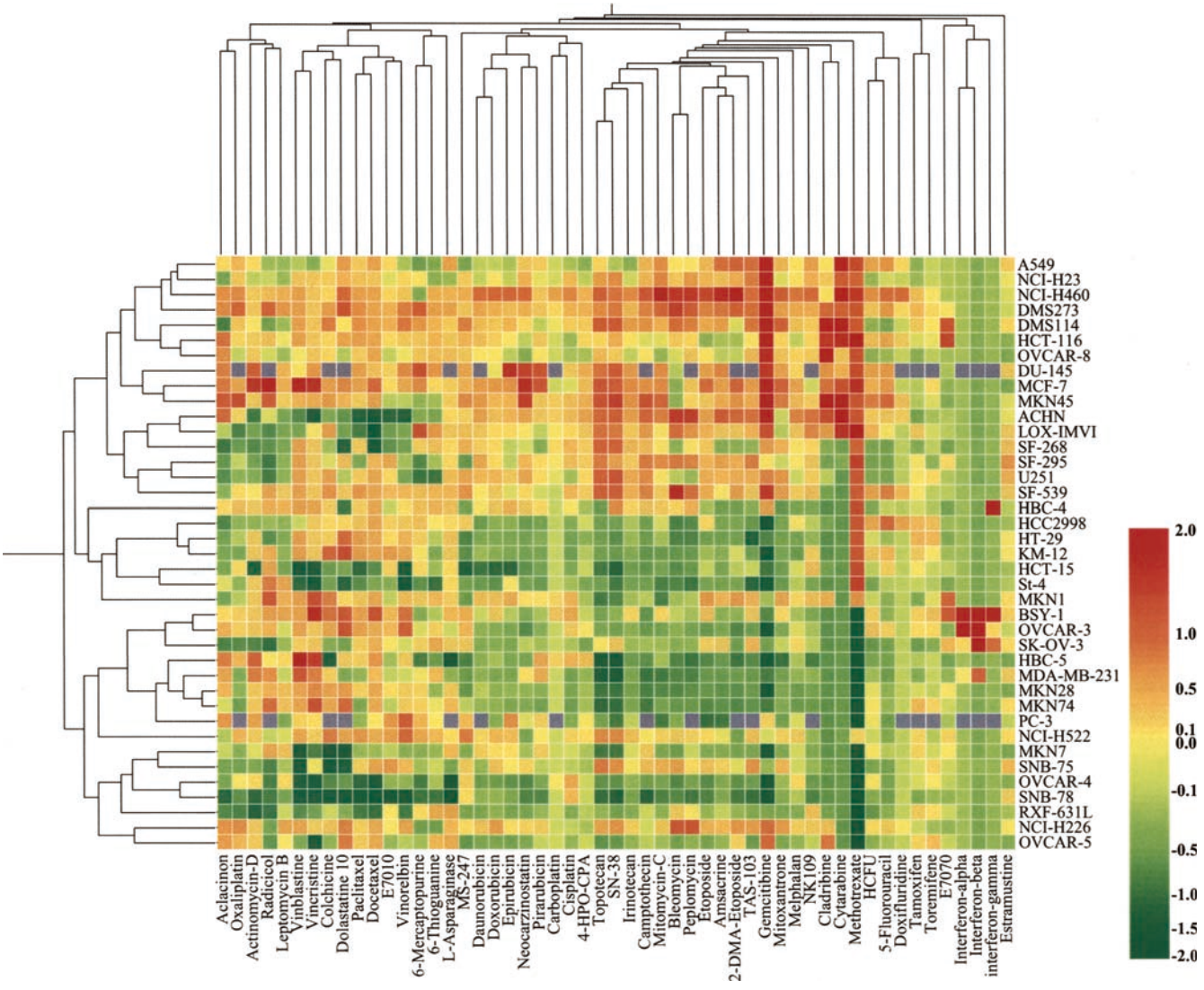

Fig. 1 Classification of anticancer drugs according to their patterns of activity against 39 human cancer cell lines. Chemosensitivity of 39 cell lines to 53 anticancer drugs, measured by GI_{50}, is visualized by gradient color. Gradient color indicates the difference in $|\log_{10}GI_{50}|$ from the mean $|\log_{10}GI_{50}|$ for each drug, e.g. the cell line with 2.0 (in *red*) is 100-fold more sensitive than the mean. A green point represents the opposite, and a yellow point shows the mean. A hierarchical clustering method was applied to the chemosensitivity data. The absolute values ($|\log_{10}GI_{50}|$) were used for correlation analysis. Correlations were assessed by the Pearson correlation coefficient as described previously [9, 15]. Analyses were performed using Gene Spring software (Silicon Genetics, Redwood City, Calif.). Drugs that share modes of action or targets tend to form a cluster

in nude mice, including lung, colon, stomach, breast, and ovarian cancers [15].

The natural product thiazinotrienomycin B (Fig. 2) inhibited cell growth at a low concentration (< 0.1 μM), and clearly suppressed the growth of a BSY-1 xenograft in nude mice. The results of COMPARE analysis suggested that thiazinotrienomycin B is not likely to be a conventional anticancer drug such as known tubulin binders (vinca alkaloids and taxanes) [6]. However, thiazinotrienomycin B had an inhibitory effect on tubulin polymerization and may have a unique mode of action.

Another natural product, β-hydroxyisovalerylshikonin (Fig. 2), a component of the oriental herb *Lithospermum erythrorhizon*, showed a unique fingerprint. Subsequent study revealed that β-hydroxyisovalerylshikonin is a potent inhibitor of protein tyrosine kinase. It strongly inhibits the tyrosine kinase activities of epidermal growth factor (EGF) receptor and v-Src. In addition, it inhibits the tyrosine kinase of KDR/FLK-1, a type of vascular endothelial growth factor receptor [5]. A structurally related compound, shikonin, is weaker than β-hydroxyisovalerylshikonin in inhibiting the tyrosine kinase of the EGF receptor and v-Src [5]. Chemical modification of shikonin may therefore produce various tyrosine kinase inhibitors with different target specificities. These examples demonstrate that our cell-line panel is a powerful tool for identifying anticancer compounds with unique modes of action.

A second application of the cell-line panel is to enable the in silico screening of compounds with specific

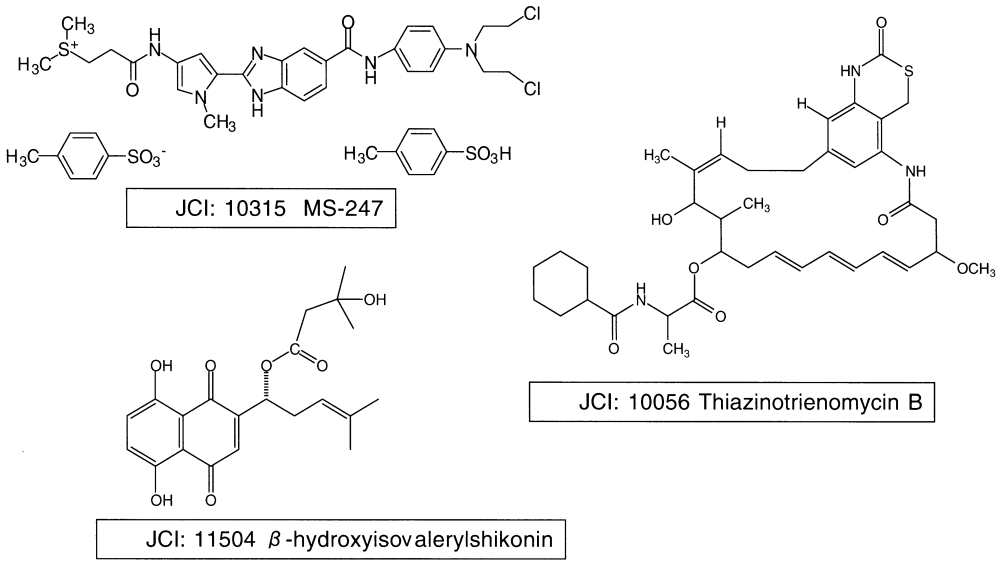

Fig. 2 Chemical structures of compounds with unique modes of action. MS-247 inhibits topoisomerases I and II. Thiazinotrienomycin B is not likely to be a conventional anticancer drug; its mode of action is unknown. β-Hydroxyisovalerylshikonin strongly inhibits the tyrosine kinase activity of the EGF receptor and v-Src. It also inhibits the tyrosine kinase of KDR/FLK-1

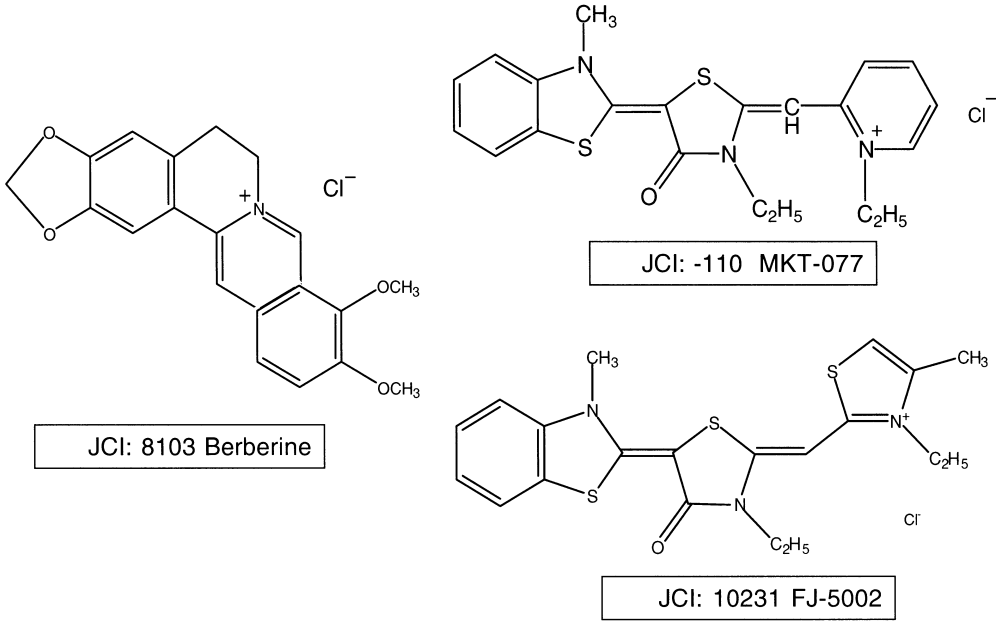

Fig. 3 Chemical structures of telomerase inhibitors. MKT-077 was extracted by in silico screening using berberine as a seed for the COMPARE analysis, then FJ-5002 was extracted by a second round of COMPARE analysis using MKT-077. The order of telomerase inhibitory activity is FJ-5002 > MKT-077 > > berberine

pharmacological activities. The database of our cell-line panel holds information on more than 2000 compounds and is thus likely to include a range of compounds with a wide variety of unknown pharmacological activities. If we have a seed compound with a weak but desirable pharmacological activity, we may encounter candidates for more potent compounds in the database using COMPARE analysis, as in our identification of the potent telomerase inhibitor FJ-5002 (Fig. 3) [8]. At the primary screening, we identified an alkaloid berberine (Fig. 3) as a moderate inhibitor of telomerase. Using this alkaloid as a seed compound in the COMPARE analysis, we extracted berberine-like compounds and mitochondria-accumulating agents that are highly related to berberine. Among these compounds, MKT-077 (Fig. 3), a rhodacyanine derivative, showed a stronger inhibitory activity than berberine. With MKT-077 as an upgraded seed for another round of COMPARE analysis, we identified the rhodacyanine FJ-5002, a close derivative of MKT-077, as the most potent telomerase inhibitor. These results indicate that in silico screening with COMPARE analysis may facilitate the search for candidate compounds with desirable pharmacological activity.

Chemosensitivity and gene expression profile

Chemotherapeutic drugs induce cell-cycle arrest and cell death in cancer cells through various pathways. More than 10,000 genes are expressed in the cancer cell, so a series of gene products on the drug-related pathways, rather than a single target molecule, determine cellular chemosensitivity.

To explore genes that predict or determine the chemosensitivity of cancer cells, we investigated the expression of 9216 genes in the 39 human cancer cell lines using cDNA microarrays [3]. We combined the gene expression database with the chemosensitivity database described above and used Pearson correlation analysis to identify genes with expression patterns that show significant correlation to chemosensitivity patterns. Scherf and colleagues first described this methodology using the NCI 60 cell-line panel [11].

We used our 39 cell-line panel and chemosensitivity data for 55 anticancer drugs to identify more than 1000 genes that satisfied the criteria described below. We determined the degree of interdependence between drug activity and gene expression pattern by calculating Pearson correlation coefficients using the formula $r = [\Sigma(x_i - x_m)(y_i - y_m)]/[\Sigma(x_i - x_m)^2 \Sigma(y_i - y_m)^2]^{1/2}$, where x_i represents the log expression ratio ($\log_2$Cy5/Cy3) of gene x in cell i, and y_i the log sensitivity ($|\log_{10}GI_{50}|$) of cell i to drug y; x_m represents the mean of the log expression ratio of gene x, and y_m the mean sensitivity ($|\log_{10}GI_{50}|$) of the drug. In this analysis, we selected genes that passed the cutoff filter: signal intensities greater than 25,000 relative fluorescence units or signal–noise ratios above 3 in either Cy3 or Cy5 in more than 80% of cases (i.e. 32 of 39 cell lines examined). We then selected genes with expression patterns that showed significant correlation to drug activity patterns. A significant correlation was defined as $P < 0.05$ and a slope of the regression line greater than 1.5, where the difference of the $|\log_{10} GI_{50}|$ values between the most- and least-sensitive cell lines was fixed as 1. We identified 1071 genes that passed these selection criteria for at least one of the 55 drugs.

Some genes commonly correlated with various classes of anticancer drug while other genes correlated only with specific drugs with similar mechanisms of action. The latter group of genes may reflect the efficacy of each class of drugs.

Some known correlations were confirmed. For example, we observed a positive correlation between DNA topoisomerase II α and β expression and sensitivity to etoposide (a DNA topoisomerase II inhibitor), and a negative correlation between thymidylate synthetase expression and 5-fluorouracil sensitivity. On the other hand, many unexpected genes were identified. The expression of *AKR1B1* and *DDB2* correlated positively, and that of *LIMK2* and *CTSH* negatively, with sensitivities to various types of drugs [3]. The statistical validation of our findings using a number of other cell lines and the investigation of a causal relationship between gene expression and chemosensitivity are underway.

Throughout the above analysis, the Pearson correlation coefficients were not high, which is reasonable considering that the chemosensitivity of cancer cells does not appear to be determined by the expression of a single gene. Development of other data-mining methodologies to analyze teams of genes on the chemosensitivity pathway is important. It is also important to determine gene expression changes induced by drug administration [2, 16]. These studies could lead to the discovery of predictive markers of chemosensitivity as well as new targets of cancer chemotherapy.

Conclusions

The panel of 39 human cancer cell lines and its database have proved useful in discovering drugs and investigating the relationship between chemosensitivity and gene expression. This panel, as well as that of the NCI, can provide rich and fundamental information on the pharmacological modes of chemicals and on variations in cancer. An expansion of the database and progress in data-mining methodology will result in the panel of human cancer cell lines being a more fertile soil for producing multiple approaches in cancer chemotherapy.

Acknowledgements I would like to thank Dr. Robert Shoemaker of the Developmental Therapeutics Program, NCI, for his generous help in starting the human cancer cell-line panel system in Japan. I appreciate the collaboration of the members of Screening Committee of New Anticancer Agents supported by grant-in-aid for scientific research on priority areas from the Ministry of Education, Culture, Sports, Science and Technology, Japan. I also thank Ms. Kanami Yamazaki for her assistance in operating the human cancer cell-line system.

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