Identification of Candidate Genes Determining Chemosensitivity to Anti-cancer Drugs of Gastric Cancer Cell Lines

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In order to efficiently develop improved cancer therapies it is important to predict the efficacy of anti-cancer drugs. In this regard, identification of genes that are related to drug sensitivity is vital. We previously established a panel of 39 human cancer cell lines (JFCR39) and a panel aiming for organ-specific analysis of 45 human cancer cell lines (JFCR45). Here, we focus on 20 human gastric cancer cell lines from JFCR45, a panel of human cancer cell lines to predict genes that determine chemosensitivity to anti-cancer drugs. We measured both chemosensitivity to a range of anti-cancer drugs as well as changes in gene expression profile. We then identified genes in which expression is related to chemosensitivity by using a Pearson correlation. As a result, anti-cancer drugs that have similar mechanisms of action showed similar fingerprints in the JFCR45. Furthermore, we identified many candidate genes related to the sensitivity of gastric cancer cells to anti-cancer drugs.

Key words gastric cancer; microarray; cancer cell line panel; chemosensitivity; JFCR45

Gastric cancer is the fourth most common cancer worldwide, with 934000 new diagnoses per annum, and the second most common cause of cancer-related death, with 700000 deaths annually. Unfortunately, the use of chemotherapy to treat gastric cancer has no established standard of care, though TS-1 is mainly used in Japan. Examples of drugs used in the treatment of gastric cancer include 5-fluorouracil (5-FU), cisplatin (CDDP), paclitaxel, doxorubicin and methotrexate (MTX), or their derivatives in single-agent chemotherapy or various combination drug therapy. Effective chemotherapy with relatively few side effects is required. In order to efficiently develop a successful chemotherapeutic regimen, it is important to identify sensitive and resistant genes to the anti-cancer drugs. Genes determining sensitivity of cancer to anti-cancer drugs are useful for predicting drug efficacy in individual patients and for the discovery of new targets for anti-cancer drugs. Suppression in the function of resistant genes, or their coded proteins, may make it possible to enhance drug sensitivity.

JFCR39, a panels of 39 human cancer cell lines, was developed to screen and evaluate anti-cancer agents. In addition, JFCR45 has developed aiming for the organ-specific analysis and consists of 45 human cancer cell lines derived from tumors from three different organs: breast, liver, and stomach. We have previously demonstrated that both JFCR39 and JFCR45 systems can be used to predict the molecular targets or evaluate the mechanism of action of the test compounds by comparing their cell growth inhibition profiles (i.e., fingerprints). In addition, we also used correlation analysis to explore the genes related to chemosensitivity of JFCR45.

MATERIALS AND METHODS

Cell Cultures In this study, we used 20 gastric cancer cell lines; St-4, MKN1, MKN7, MKN28, MKN45, MKN74, GCY, GT3TKB, HGC27, AZ521, ISt-4, NUGC-3, NUGC-3/5FU, HCS-42, AGS, KWS-1, TGS-11, OKIBA, ISt-1 and AOTO in the JFCR45. All cell lines were cultured in RPMI1640 (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 5% fetal bovine serum, penicillin (100 units/ml) and streptomycin (100 mg/ml) in humidified air containing 5% CO₂ at 37 °C.

Determination of Cell Growth Inhibition Profiles Growth inhibition was measured by determining changes in the amount of total cellular protein after 48 h of drug treatment using a sulforhodamine B assay. The GI₅₀ values, which represent 50% growth inhibition concentration, were evaluated as described previously. Several experiments were performed to determine the median GI₅₀ value for each drug. Absolute values were then log-transformed for further analysis.

Anti-cancer Drugs Methotrexate, docetaxel and oxaliplatin (I-HP) were obtained from Wyeth Lederie Japan (Tokyo, Japan), Aventis Pharma (Strasbourg, France) and Asahi Kasei (Tokyo, Japan), respectively. Carboplatin,
CDDP, and paclitaxel were obtained from Bristol-Myers (New York, NY, U.S.A.).

Identification of Gene Expression Profiles by cDNA Microarray  
Expression profiles of 3537 genes in 20 human gastric cancer cell lines were examined using Atlas™ human 3.6 arrays (Clontech, Palo Alto, CA, U.S.A.) according to the manufacturer's instructions. Each gene signal was normalized by the 90th percentile of all signals on each membrane. The genes, whose expression levels differed more than two times between duplicates, were eliminated. We then calculated the average values of the two experiments for all the remaining genes. The cut-off value was 0.3-fold of the 90th percentile and the gene signals were replaced with 0.3. All values were log-transformed (base 2).

Correlation Analysis between the Gene Expression and Chemosensitivity Profiles  
The genes, whose expressions were observed in over 50% of all cell lines examined, were selected for correlation analysis. The degree of similarity between the chemosensitivity and gene expression were calculated using the Pearson correlation coefficient.

RESULTS

Chemosensitivity of the anti-cancer drugs to the 20 gastric cancer cell lines was determined as described in Materials and Methods. The concentration of drug causing 50% cell growth inhibition was represented as GI$_{50}$. Figure 1 shows the fingerprints of six anti-cancer drugs: MTX, l-OHP, carboplatin, CDDP, paclitaxel and docetaxel.

In the gastric cancer cell lines, drugs acting via a similar mechanism display similar fingerprints. For example, Pearson correlation coefficient ($r$) between paclitaxel and docetaxel, both of which are tubulin binders, was 0.920. Similarly, the $r$ between CDDP and carboplatin, both of which are alkylating reagents, was 0.776. In contrast, the $r$ between paclitaxel and CDDP, whose mechanisms are different, was low (0.363). Similarly, MTX display the fingerprint different from those of other drugs. On the other hands, l-OHP, which is the same alkylating reagents as CDDP and carboplatin, shows no similarity to them.

Next, we performed Pearson correlation analysis of the gene expression and chemosensitivity. As a result, many genes whose expressions were correlated with respect to the sensitivity of each drug were identified (Table 1). IRF9 (interferon regulatory factor 9), RAD23A, YWHAZ (14-3-3 zeta), and LAMP2 (lysosomal-associated membrane protein 2) were commonly extracted from CDDP (Table 1c) and carboplatin (Table 1d) sharing the same mechanism of actions; i.e. a platinum-based drugs causing crosslinking of DNA. Though l-OHP (Table 1b) is also a platinum-based drugs, no common genes was extracted except M6PR (46 kDa mannose 6-phosphate receptor). SLC6A8 (Solute carrier family 6 member 8; creatine transporter 1), PITPNA (phosphatidylinositol transfer protein), RAB11B, NAP1L1 (nucleosome assembly protein 1-like 1), and PYCR1 (pyrroline-5-carboxylate reductase 1) were also commonly extracted from tubulin binders, paclitaxel (Table 1e) and docetaxel (Table 1f). Surprisingly, RASA1 (RAS p21 protein activator 1), RPS9, RAB28, GNA12, NAP1L1, SERPINF2 (serine proteinase inhibitor, clade F, alpha-2), and KDELRE2 (KDEL receptor 2) from MTX (Table 1a) were commonly extracted from paclitaxel and/or docetaxel having different mechanisms of actions.

On the others hands, we obtained the genes, which are thought to be functionally related to the mechanisms of actions. For example, genes related to platinum-based drugs, CDDP (Table 1c) or carboplatin (Table 1d) involve RAD23A, APEX1 (AP endonuclease 1) and ID2 (inhibitor of DNA binding 2), which are related to DNA damage. HES1 (hairy and enhancer of split 1), YWHAZ and IRF9 were also related to CDDP or carboplatin. These genes and/or their coded proteins have been reported to be related to p53. Paclitaxel (Table 1e) and docetaxel (Table 1f) are highly correlated to the expression of TUBA1C (tubulin, alpha 1c) and ACTB (beta actin) genes, respectively. Interestingly, the action of these drugs is also correlated to the small G protein family and their relevant genes, such as RASA1 (RAS p21 protein activator 1), RANGAP1 (Ran GTPase activating protein 1), RAB11B, RAB28 and GNA12 (GTP-binding regulatory protein Gi alpha-2 chain).
Table 1. Genes Related to the Sensitivity to MTX, l-OHP, CDDP, Carboplatin, Paclitaxel, and Docetaxel

<table>
<thead>
<tr>
<th>a. MTX</th>
<th>b. l-OHP</th>
<th>c. CDDP</th>
<th>d. Carboplatin</th>
<th>e. Paclitaxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rank</td>
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<td>r</td>
<td>Symbol</td>
<td>Rank</td>
</tr>
<tr>
<td>1</td>
<td>3-A03f</td>
<td>0.787</td>
<td>ENTPD2</td>
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<tr>
<td>2</td>
<td>2-B11f</td>
<td>0.762</td>
<td>RASA1</td>
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</tr>
<tr>
<td>3</td>
<td>2-B10f</td>
<td>0.747</td>
<td>NPM1</td>
<td>3</td>
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<tr>
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<td>0.730</td>
<td>RPS9</td>
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<tr>
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<td>0.715</td>
<td>SERPINB10</td>
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<tr>
<td>6</td>
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<td>0.675</td>
<td>PLK1</td>
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<td>PPFK</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>3-D11j</td>
<td>0.652</td>
<td>SARS</td>
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<td>POLR2H</td>
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<td>2-E13k</td>
<td>0.607</td>
<td>GNAI2</td>
<td>16</td>
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</table>

‘Gene ID’, ‘r’, and ‘Symbol’ show the manufacturer’s gene ID, Pearson correlation coefficient between chemosensitivity to drugs and gene expression, and the name of the gene according to HUGO database, respectively. Bold indicated the commonly extracted gene from two drugs sharing the same mechanisms of actions; i.e. from 1-OHP and CDDP, l-OHP and carboplatin, CDDP and carboplatin, paclitaxel and docetaxel. Italic indicated the commonly extracted gene from two drugs having the different mode of actions. For example, CAPN1 from MTX and l-OHP was shown in italic.

DISCUSSION

We previously established panels of human cancer cell lines, JFCR39 and JFCR45, as a means of predicting the pharmacological mechanism of anti-cancer agents. We represented that the panel of human cancer cell lines are useful for the identification of genes sensitive/ or resistant to anti-cancer drugs, according to identifying two genes, HSPA1A (heat shock 70 kDa protein 1A) and JUN (c-Jun), which determined sensitivity to mitomycin C using JFCR45. In this study, we performed the analysis based on the derived-organ using a gastric subpanel of human cancer cell lines.

In the gastric subpanel of human cancer cell lines, drugs displaying a similar mechanism of action show a similar fingerprint, as was the case with JFCR39 and JFCR45. This result indicates that the methodology, used for JFCR39 and JFCR45, to extract the sensitive or resistant genes to the anti-cancer drugs may have applicability to the gastric subpanel of human cancer cell lines. Interestingly, CDDP and l-OHP, platinum-based drugs, which bind and cause cross-linking of DNA, display different fingerprints. This result may reflect a difference of application, efficacy and side effects of the two platinum-based drugs. Indeed, these observations are consistent with the fact that no genes were commonly extracted by CDDP and l-OHP.

Furthermore, we performed Pearson correlation analysis of the gene expression and the chemosensitivity to extract candidate genes that were related to the chemosensitivities of the anti-cancer drugs. As a result, genes related to platinum-based drugs, CDDP or carboplatin, involve RAD23A gene. RAD23A is one of two human homologs of Saccharomyces cerevisiae Rad23 and plays a role in DNA damage recognition in base excision repair. It is possible that the elevated expression of RAD23A makes tumor cells more resistant to DNA damage by CDDP and carboplatin. Paclitaxel and docetaxel bind to tubulin and prevent mitosis through microtubule over-stabilization. Genes related to these drugs involve cytoskeleton genes such as tubulin or actin family.
gene. Small G protein family genes and genes related to them, which regulate cytoskeleton reconstitution and vesicular transport,\textsuperscript{15} were also extracted as potential candidates. Surprisingly, seven genes from MTX were commonly extracted from paclitaxel and/or docetaxel having different mechanisms of actions. This result indicates that there are some common mechanisms of action except main mechanisms of actions related to their drug efficacy. Our results suggest that genes whose expression is correlated to the sensitivity of each drug display a related mechanism of action, although there is an exception. These extracted genes may be useful for predicting drug efficacy and for overcoming drug resistance. However, the relationships between chemosensitivity and expression of certain genes need to be validated by quantitative polymerase chain reaction (PCR). Moreover, it is important to establish that the modulation of gene expression levels induce a change of chemosensitivity.

In conclusion, the present study indicates that drugs showing a similar mechanism of action share a comparable fingerprint in gastric cancer cell lines, as was shown for JFCR39 and JFCR45 cell line panels. Furthermore, we present candidate genes whose expression is related to the sensitivity of gastric cancer cells to anti-cancer drugs.

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