Genes Potentially Associated with Resistance of Lung Cancer Cells to Paclitaxel


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Paclitaxel is a natural substance from the group of taxanes and one of the most widely used drugs in chemotherapy of malignant tumors, including the non-small cell lung cancer (NSCLC). Treatment of patients with advanced NSCLC with paclitaxel alone or in combination with other drugs led to complete or partial tumor regression in at most 41% of patients [1]. Individual selection of drugs that are effective for a particular patient is one of the hottest approaches in the strategy to improve the results of chemotherapeutic treatment of cancer patients. This work is dedicated to finding new markers and studying the resistance mechanisms of lung cancer to paclitaxel.

The main mechanism of action of paclitaxel is the blockade of cell division due to its specific binding to β-3 tubulin and stabilization of microtubules. An increased expression of some β–tubulin isotypes correlates with resistance to paclitaxel and the degree of malignancy of tumors of the lung, prostate, ovary and other tissues. The same result can be caused by mutations of β-tubulins, although the clinical significance of mutations is controversial (see reviews [2, 3]).

It is known that the resistance of tumor cells to paclitaxel may be associated with an increased expression or polymorphism of some ABC-transporters responsible for multidrug resistance [4] as well as with activity of growth factor receptors (EGFR and HER-2), proteins LIMK1, LIMK2, TGFB1, STMN1, spindle checkpoint proteins, apoptosis regulators, and others [2, 3].

The use of DNA microchips [5–10] made it possible to reveal the sets of genes whose expression correlates with chemoresistance of lung cancer cells. However, the sets of genes that, with a certain probability, may be involved in resistance development, which were proposed by different authors, differ. In addition, the genes detected in cell cultures may be uninformative for predicting the response of patients to chemotherapy [11]. At the same time, studies of clinical specimens only may give ambiguous results due to polymorphisms, which lead to a high interindividual variability of systems involved in the development of chemoresistance of cancer patients, as well as because of chemotherapeutic regimens that usually include several drugs.

To search for new informative markers and to reveal the molecular mechanisms of drug resistance in tumors, we studied the relationship between the sensitivity of lung cancer cells to paclitaxel and the expression of a wide range of genes using the new microchip platform Affymetrix Human GeneChip ST1.0, which contained probes for over 28000 human mRNAs.

As a biological model, we used lung cancer cells of six lines derived from ATCC (A549, NCI-H292, NCI-H460, and NCI-H1299) and ECACC (NCI-H322 and NCI-H358). Although the sensitivity of certain lines to various drugs is known (http://discover.nci.nih.gov/cellminer/), we redefined the IC50 of paclitaxel for all cells using the MTT technique [12] in order to eliminate the effect of subculturing of cells on their sensitivity to the drug. For each cell line, we analyzed the results of at least three significant measurements (Fig. 1).

Hybridization of labeled samples prepared from total cell RNA with microchips was performed using the equipment and method of Affymetrix. The results of hybridization were processed by the RMA algorithm using the xps library (Christian Stratowa; http://www.bioconductor.org) in R system. Fluorescence signals were filtered using I/INI algorithm [13]; signals with random variation were ignored.
Cells of all six lines with database (http://www.geneontology.org), and the Pathway Interaction Databases (NCI PID, http://pid.nci.nih.gov/index.shtml), Gene Ontology for this purpose, we identified the biological processes that involve the identified genes using NCI. For this purpose, we identified the biological processes that involve the identified genes using NCI. For this purpose, we identified the biological processes that involve the identified genes using NCI. For this purpose, we identified the biological processes that involve the identified genes using NCI. For this purpose, we identified the biological processes that involve the identified genes using NCI. For this purpose, we identified the biological processes that involve the identified genes using NCI. For this purpose, we identified the biological processes that involve the identified genes using NCI. For this purpose, we identified the biological processes that involve the identified genes using NCI. For this purpose, we identified the biological processes that involve the identified genes using NCI.

Table 1. Genes differing in the expression level between groups of resistant and sensitive cells*

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<tr>
<th>Gene predominantly expressed in resistant cells</th>
<th>Gene predominantly expressed in sensitive cells</th>
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<tr>
<td>XAGE1D, FLJ35848, UCP2, VCAN, PEG10, GEM, TNNT1, ARL4C, CCDC144A, C8orf37</td>
<td>SERPINB2, CDH13, NID2, IL1A, SORBS2, FAM134B, C4orf18, ASS1, SMOC1, ADAMTS1</td>
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* Ten genes that most significantly differed in the level of expression are shown.

According to the results of IC50 measurements, cells were divided into three groups: resistant cells (lines A549, NCI-H1299, and NCI-H358), cells of intermediate sensitivity (line H322), and sensitive cells (lines NCI-H292 and NCI-H460). According to the results of hybridization with the microchip, we selected the probes for which the difference between the threshold fluorescence values (expression index) in the first and third groups differed by at least twice (Table 1). In addition, we determined the Pearson correlation coefficient \( R \) for the gene expression rate in cells of all six lines with IC50 and selected genes with \( R \geq 0.9 \) and \( R \leq -0.9 \). Having combined the genes selected by the two methods, we obtained two sets of genes whose expression is associated with paclitaxel-resistant (56 genes) and paclitaxel-sensitive (52 genes) phenotypes.

The obtained panels of genes were used to predict possible mechanisms that determine the sensitivity or resistance of tumor cells to paclitaxel. For this purpose, we identified the biological processes that involve the identified genes using NCI. Pathway Interaction Databases (NCI PID, http://pid.nci.nih.gov/index.shtml), Gene Ontology database (http://www.geneontology.org), and the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/kegg2.html) (Table 2).

The study of the processes showed that the resistant cells actively expressed the genes that are involved in signal transduction cascades mediated by SMAD2/3, TGF-\( \beta \), and C-MYC. These signaling pathways are responsible for the passage of cell-cycle checkpoints by the cell in the G1 phase and are not associated with interleukins. Probably, these genes and signal transduction pathways allow cells to stop in the G1 phase and survive at low concentrations of paclitaxel [14]. Note an increased expression of certain target genes of C-MYC, a transcriptional regulator of a broad spectrum of genes that control pivotal cell functions, including DNA replication and repair and the cell cycle passage. In addition, paclitaxel-resistant cells are characterized by an increased expression of some genes whose products are involved in signal transduction and genes encoding proteins with different transport functions: ENY2 (a transcription activator that ensures the transport of mRNA to the cytoplasm), SMAD3 (protein involved in nuclear translocation of \( \beta \)-catenin), GABRB3 (a member of the GABA-A family receptors, which are components of ion channel), and UCP2 (mitochondrial regulator of proton transport). The specific role of these processes in drug resistance of cells should be investigated in further studies.

An increased expression of genes encoding interleukins and other regulators of apoptosis was detected in the paclitaxel-sensitive cells. This indicates a possible role of interleukin-dependent signaling cascades in cell response to paclitaxel and their relation to certain diseases and interleukin-mediated reactions of the body (e.g., prion diseases, inflammation, etc.). This opens up new ways to boost cell response to the drug. In particular, it was shown that the prion protein PrPε interacts with P-glycoprotein during the development of multidrug resistance and that it is involved in the regulation of doxorubicin-induced apoptosis by modulating the expression of Bel-2 and Bax genes [15]. Interestingly, the group of genes identified in the paclitaxel-sensitive cells includes both the genes preventing cell proliferation (ADAMTS1 and CDH13) and the genes with the opposite function, which inhibit apoptosis (SERPINB2/PAI-2 and SERPINB9/PI-9). Apparently, the paclitaxel-sensitive cells also differ from the paclitaxel-resistant cells in some metabolic characteristics (oxidation–reduction, fatty acid metabolism, and the respiratory electron transport chain), which should also be studied in the future.

One of the key regulators of the cell cycle and apoptosis that may determine the degree of cell resistance to drugs, in particular to antimitotic drugs, is p53 [3]. We noted that the group of paclitaxel-sensitive cell lines included the cell lines with the wild-type p53 gene (p53wt, lines NCI-H292 and NCI-H460) and that the group of paclitaxel-resistant cell lines included two cell lines with the p53 gene deletion.