Prediction of chemotherapeutic resistance in serous ovarian cancer with low-density custom microarray

Hong-Lan Zhu[1], Hao-Xia Zeng[2], Xu-Dong Liang[1], Xiao-Hong Chang[1], Jian-Liu Wang[1], Heng Cui[1], Li-Hui Wei[1]

[1]Department of Obstetrics and Gynecology, Peking University People's Hospital, Beijing 100044, China;
[2]Department of Obstetrics and Gynecology, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China.

To the Editor: Ovarian cancer is the leading cause of death from gynecologic malignancies. The major limitation to the successful long-term treatment of ovarian cancer is the development of chemotherapeutic resistance, which involves thousands of genes. In this study, a single-channel low-density custom microarray was designed to compare gene expression profiles between chemotherapy-resistant and chemotherapy-sensitive ovarian cancer. The 87 genes were identified to be differentially expressed in resistant and sensitive specimens that can be used to fairly accurately predict drug resistance in serous ovarian cancer.

cDNA microarrays to research chemotherapy resistance in serous ovarian cancer were customized with use of Agilent Technologies' (Santa Clara, CA, USA) eArray 5.0 program. Each customized microarray contained 1366 genes, which were chosen through two sources. The first source was the Gynecologic Oncology Center at our hospital, which had found 95 genes associated with chemotherapy resistance in ovarian cancer with use of a whole genomic gene chip. The second source was from PubMed, HighWire, Elsevier, ProQuest, BlackWell, CNKI, and similar resources that we took full advantage of for document retrieval.

Our study involved 23 patients with advanced serous ovarian cancer who were admitted to our hospital between January 1, 2001, and December 31, 2008. On the basis of the disease-free interval (DFI), the patients were divided into two groups: group R (Response; sensitive to chemotherapy; $n = 11$ patients; DFI > 12 months, median DFI = 27.36 months) and group N (No response; resistant to chemotherapy; $n = 12$ patients; DFI < 6 months, median DFI = 3.25 months). We chose six patients randomly from group R (R1, R3, R5, R9, R11, and R12) and group N (N2, N4, N5, N8, N11, and N12) respectively, which were used to screen for differentially expressed genes belonging to training teams. The rest (R2, R4, R6, R7, R8; N1, N3, N6, N7, N9, and N10) belonged to testing teams, which were used to test the accuracy of the model for predicting drug resistance.

Total RNA was extracted by using TRIZOL Reagent (Cat#15596-018, Life Technologies, Carlsbad, CA, USA) and checked for a RNA integrity number (RIN) number with which to inspect RNA integration by an Agilent Bioanalyzer 2100 (Agilent Technologies). Each slide was hybridized with 1.65 μg of Cy3-labeled cRNA with use of a Gene Expression Hybridization kit (Cat#5188-5242; Agilent Technologies) in a Hybridization Oven (Cat#G2545A; Agilent Technologies).

Data were analyzed with Gene Spring Software 11.0 (Agilent Technologies) and SAS (SBC Analysis System), which used R-software (The R Project for Statistical Computing) for the statistical analysis.

A total of 87 differential genes [Supplementary Table 1, http://links.lww.com/CM9/A185 and Table 2, http://links.lww.com/CM9/A186] were selected, of which 71 genes were up-regulated (N/R > 2, $P < 0.05$) and 16 genes down-regulated (N/R < 0.5) in chemotherapy-resistant patients compared with chemotherapy-sensitive patients. These genes are located on chromosome 1-22, none on the sex chromosome. It is worth noting that chromosome 16q includes seven differential genes coding metallothionein (MT2A, MT1L, MT1E, MT1B, MT1G, MT1H, and MT1X). They were all up-regulated in group N (chemotherapy-resistant) patients. The Gene Ontology (GO) enrichment analysis revealed that 87, 80, and 81 differentially expressed genes were involved with molecular function, the cellular component, and biologic processes, respectively.

We used various approaches to test the accuracy of such a prediction. One approach was hierarchical cluster analysis, which divided 11 patients (testing team of groups N

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Hong-Lan Zhu and Hao-Xia Zeng contributed equally to the work.

Correspondence to: Xu-Dong Liang, Department of Obstetrics and Gynecology, Peking University People's Hospital, Beijing 100044, China
E-Mail: liangxudong100@aliyun.com

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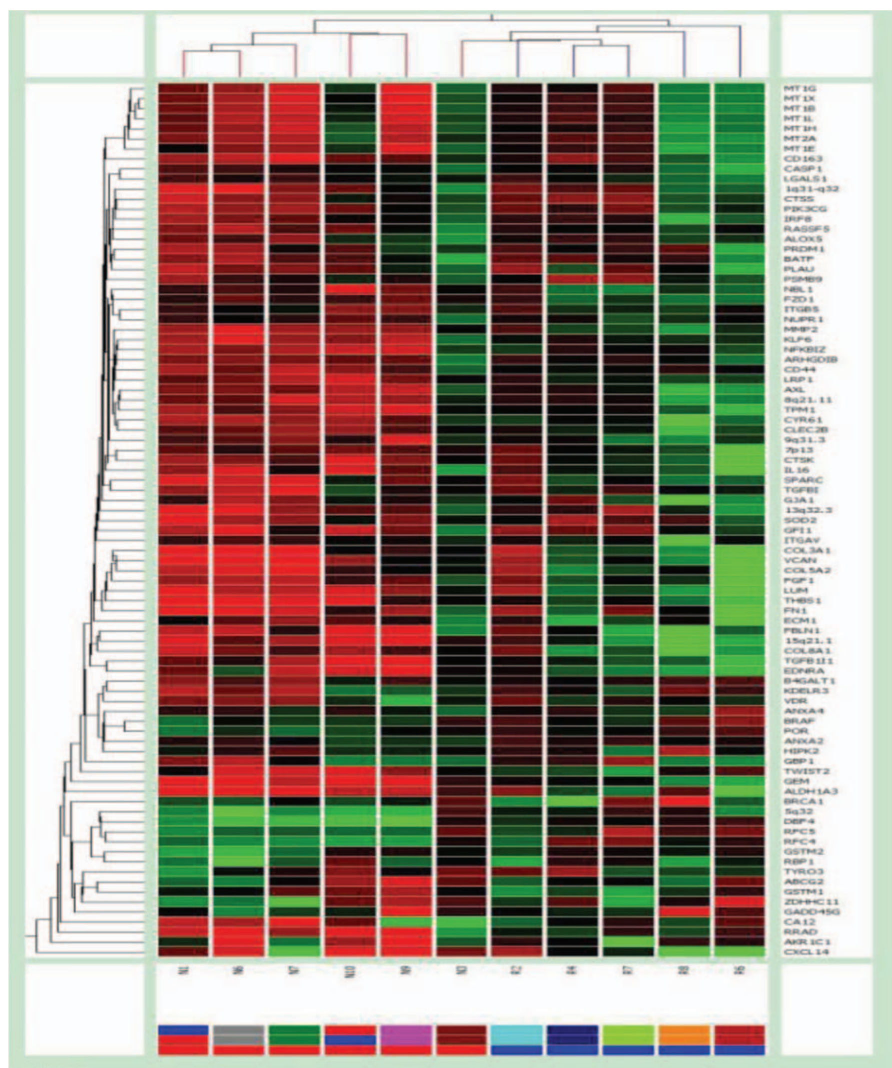

Figure 1: Hierarchical cluster analysis results from 11 testing patients.

and R) into two groups, only one resistant patient was classified in error [Figure 1], with a precision of 90.9% (10/11). Using PLS in the 11 testing patients, the 87 differential genes correctly classified 5 of 6 chemotherapy-resistant (group N) patients (83.3% sensitivity) and 4 of 5 chemotherapy-sensitive (group R) patients (80% specificity). Using SVM, the 87 differential genes correctly classified 5 of 6 chemotherapy-resistant (group N) patients (83.3% sensitivity) and 2 of 5 chemotherapy-sensitive (group R) patients (40% specificity).

With the development of microarray technology, many researchers expect to use various microarrays to screen for chemotherapy-resistant genes and to establish a prediction model for ovarian cancer. Komatsu *et al*[1] used an oligomicroarray that contained 19,981 probes to choose eight novel marker genes that alone could accurately predict progression-free survival associated with chemotherapy resistance ($r = 0.683$; $P = 0.042$).

Helleman *et al*[2] using 18K cDNA microarrays to choose nine genes (FN1, TOP2A, LBR, ASS, COL3A1, STK6, SGPP1, ITGAE, and PCNA) and construct a model for predicting platinum resistance, with a sensitivity of 89% (95% confidence interval [CI]: 0.68–1.09%) and a specificity of 59% (95% CI: 0.47–0.71%) (odds ratio = 0.09; $P = 0.026$).

In 2017, Januchowski *et al*[3] used the microarray to determine alterations in the level of expression of genes in cisplatin-, doxorubicin-, topotecan-, and paclitaxel-resistant variants and they found different gene changes related to different drug resistance.

Our study had several advantages compared with other research. To minimize the effect of histologic types on chemosensitivities, all of our patients had serous ovarian cancer. We designed and manufactured a low-density custom microarray to avoid the interference of unrelated genes. We chose tumor tissue samples from patients and not from cell lines, which can reveal original gene changes in ovarian cancer patients and avoid genes changing during cell culture. However, our study can be further improved because of our small sample size and lack of prospective verification. Maybe we can take advantage of TCGA to validate our findings.

In conclusion, we identified 87 genes that had different patterns of expression in resistant and sensitive serous ovarian cancer tissue, which can be used to predict chemotherapeutic resistance in serous ovarian cancer. On the basis of these 87 genes, we will construct a new low-density custom microarray that can accurately predict chemotherapeutic resistance and can be used to help physicians establish chemotherapy strategies. Of course, larger prospective studies must be done to ensure accuracy.

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Conflicts of interest

None.

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