THE GENE EXPRESSION PROFILE OF HIGHLY METASTATIC HUMAN OVARIAN CANCER CELL LINE BY GENE CHIP


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ABSTRACT

Objective: To study the gene expression of highly metastatic human ovarian carcinoma cell line (HO-8910PM) and to screen for novel metastasis-associated genes by cDNA microarray. Methods: The cDNA was retro-transcribed from equal quantity mRNA derived from tissues of highly metastatic ovarian carcinoma cell line and normal ovarian, and was labeled with Cy5 and Cy3 fluorescence as probes. The mixed probes were hybridized with BioDoor 4096 double dot human whole gene chip. The chip was scanned by scanArray 3000 laser scanner. The acquired image was analyzed by ImaGene 3.0 software. Results: By applying the cDNA microarray we found: A total of 323 genes whose expression level were 3 times higher or lower in HO-8910PM cell than normal ovarian epithelium cell were screened out, with 71 higher and 252 lower respectively. Among these 10 were new genes. 67 genes showed expression difference bigger than 6 times between HO-8910PM cell and normal ovarian epithelium cell, among these genes 12 were higher, 55 lower, and two new genes were found. Conclusion: cDNA microarray technique is effective in screening the differentially expressed genes between human ovarian cancer cell line gene expression profile difference will help the gene diagnosis, treatment and protection.

Key words: Ovarian cancer, High metastasis, cDNA microarray, Gene expression profile

METHODS

Materials

Highly metastatic human ovarian tumor cell line HO-8910PM was established in our institute. One \( 10^6 \) of cultured cells were collected and stored in liquid N2. Normal ovarian epithelium were taken from 11 cases (34–51 yr. Old) with other diseases. After resection, parts of the ovarian tissues were immediately stored in liquid N2. The remaining parts of the samples were provided for histopathological examination. All these ovarian tissues were identified as normal ovarian epithelial tissues.

Chip Preparation

Four thousand and ninety six target cDNA clones were used in cDNA chip (provided by United Gene Ltd. And cooperative fellows). These genes were amplified through PCR using universal primers and then purified with standard method. The quality of PCR was monitored by agarose electrophoresis. The target genes were dissolved in 3x SSC spotting solution, then spotting on silylated slides (Telechem. Inc) by Cartesian 7500 spotting Robotics (Cartesian, Inc.) Each target gene was

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dotted twice. After spotting, the slides were hydrated (2 hours), dried (0.5 hour, room temperature), and cross-linked under UV light, then treated with 0.2% SDS, H2O and 0.2% NaNBH4 for 10 min respectively. The slides were dried in the cold and ready for use.

**Probe Preparation**

Total RNA in cancer cells and in normal ovarian tissues were extracted respectively by single step method. The normal ovarian tissues and the cultured ovarian cancer cells were taken out from liquid N2, immediately ground completely while adding liquid N2 into tiny powder in ceramic mortar, and homogenized in D solution plus 1% mercaptoethanol. After centrifugation, the supernatant was extracted twice with phenol: chloroform (1: 1), then once with NaAc and acidic phenol: chloroform (5: 1). The aqueous phase was precipitated by equal volume of isopropanol. The precipitate was centrifuged and dissolved with Millie-Q H2O. After purification by LiC1 precipitating method, detection showed that the quality of purified total RNA was confirmed by UV analysis and electrophoresis. mRNAs were isolated and purified by Oligotex mRNA Midi Kit (Quagen, Inc.). The fluorescent-labeled cDNA probe was prepared through retro-transcription. The probes from normal ovarian tissues were labeled with Cy3-dUTP, and from cancer tissues with Cy5-dUTP. The probes were mixed and precipitated by ethanol, and resolved in 20μl hybridization solution (5×SSC+ 0.2% SDS).

**Hybridization and Washing**

Hybridizing probe and chip were denatured respectively at 95°C bath for 5 min. The probe was added on the chip and covered with glass. The chip was hybridized in sealed chamber at 60°C for 15–17 hour. After removing from covered glass, the slide was washed in solution of 2×SSC+0.2%SDS, 0.1×SSC+0.2% SDS and 0.1%SSC for 10 min each, respectively, then dried at room temperature.

**Fluorescent Scanning and Results Analysis**

The chip was scanned by Scan array 3000 Scanner (General Scanning Inc). The Cy3 and Cy5 overall intensity were normalized and corrected by a coefficient according to the ratios of the located 40 housekeeping genes. The acquired image was analyzed by ImeGene 3.0 Software dealt with digital computer. The intensities of fluorescent signals and each ratio of Cy3 to Cy5 were compared. The data were taken as average of the two repeated spots. The standards to screen for differentially expressed gene were as follows: (1) The absolute value of the natural logarithm of the signal ratio of Cy5/Cy3 was greater than 0.69 (gene expression change>2 fold). (2) One of the signal values of Cy3 and Cy5 was greater than 600. (3) The results of PCR were good.

**RESULTS**

**Scatter-plot of Hybridization Signals on Gene Chip**

The scatter plot was plotted with Cy3 and Cy5 fluorescent signal values. It revealed a quite disperses distribution pattern. Most of spots gathered round on almost 45° diagonal line. Blue spots showed signals difference between 0.5–2 fold. Certain of red spots distributed beyond 45° diagonal line and the other far from 45° diagonal line indicated the existence of significant change occurred in many expressed genes in highly metastatic human ovarian cancer cells. These different signals showed greater than 2 fold changes compared with normal control were(Figure 1).

**Gene Expression Pattern by Scanning Analysis**

The fluorescent scanning profile of gene expression was showed in Figure 2. Cy3 labeled cDNA probe from normal ovarian tissues and Cy5 labeled cDNA probes from human ovarian tumor line HO-8910PM were hybridized through microarray. Hybridization results obtained in parallel comparing with two gene expression patterns demonstrated: (1) Three hundred and twenty three differentially expressed genes between normal ovarian tissues and HO-8910PM were detected. Among these, 71 genes showed >3 fold up regulation, and 252 genes revealed <0.33 down regulation in the highly metastatic cancer cell line. Based on their functions, these genes might be divided into 16 groups(Table 1).

(2) Comparation of the highly metastatic human ovarian tumor cell line (HO-8910PM) with normal ovarian epithelium revealed a total of 67 genes with more than 6 times expressing difference. Among these 12 genes were up regulated (Table 2) and 55 genes were down regulated <0.17, two of them were novel genes (Table 3).

**DISCUSSION**

Ovarian cancer progression in the early stage is asymptomatic. Therefore, most ovarian cancer patients are diagnosed at an advanced stage with poor therapeutic effect. Ovarian cancer has become a kind of cancer with the highest mortality in gynecological carcinoma. The genesis of ovarian cancer is the results of a series of