—Photogravure—

Assessment of Gene Expression Profile by cDNA Microarray Analysis

Mohammad Ghazizadeh[1], Oichi Kawanami[1] and Tsutomu Araki[2]
[1]Department of Molecular Pathology, Institute of Gerontology, Nippon Medical School
[2]Department of Obstetrics and Gynecology, Nippon Medical School

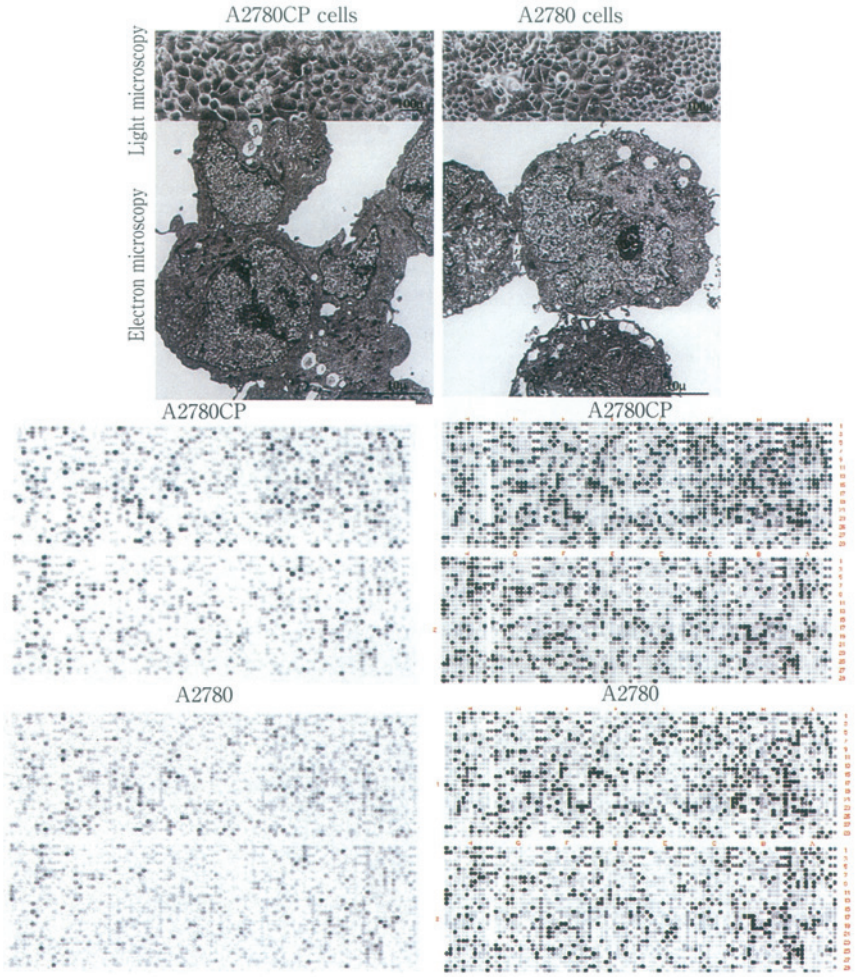

Fig. 1

With the advent of cDNA microarray technology, variations in cellular transcriptional events can now be monitored simultaneously, and expression profile at each functional state can be established and compared to one another. Here we illustrate gene expression profile analysis of a cisplatin-resistant ovarian carcinoma cell line (A2780CP) and its parental A2780 cell line, and compare them to assess changes in gene expression related to resistance to cisplatin. Equal quantities (1 μg) of RNA from the cell lines are reverse-transcribed, labeled with ^{33}P radioisotpe, and hybridized to ovary specific GeneFilters carrying more than 5,000 genes (GF223; Research Genetics, Huntsville, AL). Filter images are recorded by a phosphor-imager and analyzed comparatively using Pathways 3.0 software (Research Genetics). The upregulated and downregulated genes in cisplatin-resistant cells are identified and changes in selected genes are validated by a semiquantitative RT-PCR. This approach allows us to identify groups of genes, rather than just single genes, involved in a process, and can indicate which molecular and cellular events might be critical in such complex biological processes.

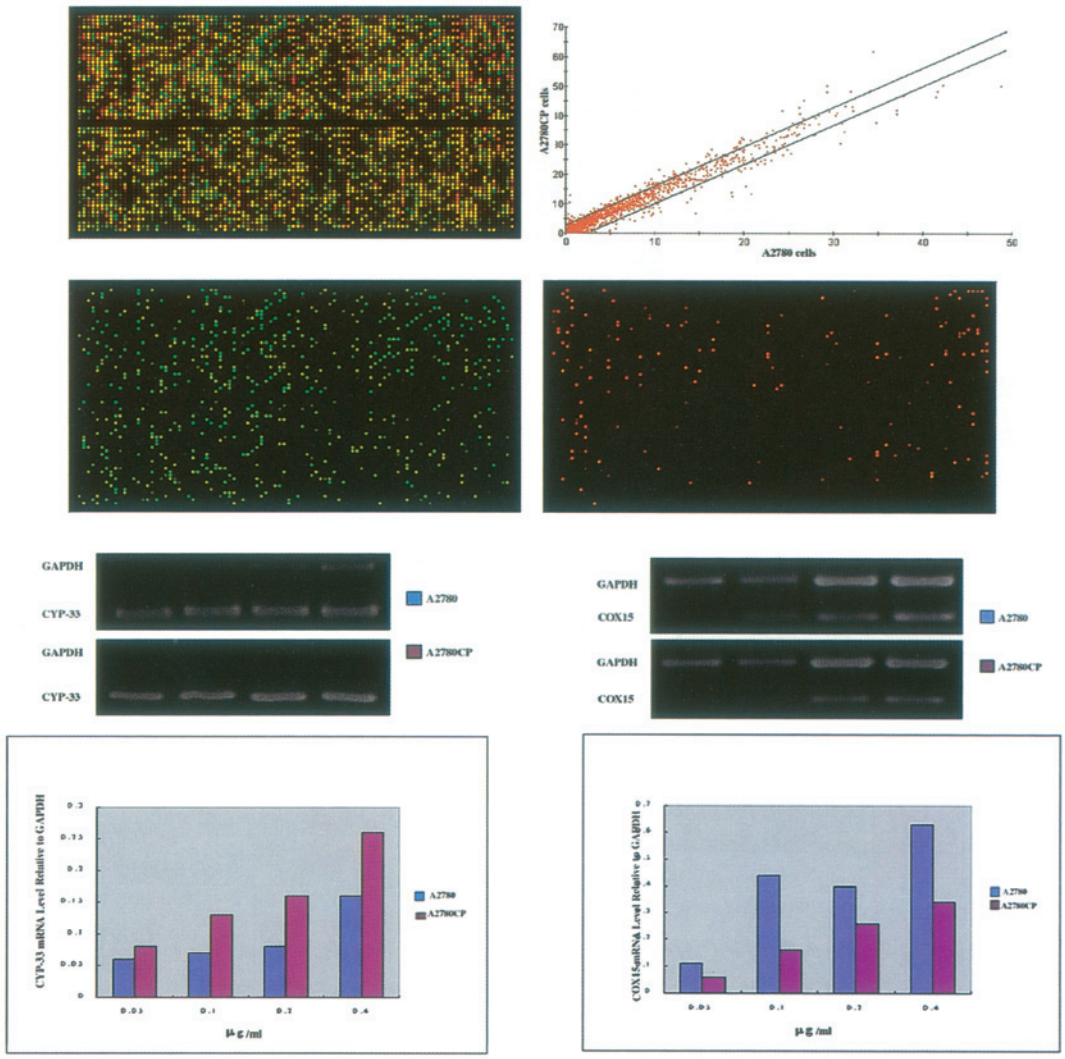

Fig. 2

Fig. 1 (*Upper*) Light and electron micrographs of cisplatin-resistant ovarian carcinoma cell line (A2780CP) and its parental cell line (A2780). No apparent morphologic difference between the two variants is observed.
(*Lower left*) Phosphor-images of cDNA microarray GeneFilters for the two cell variants after hybridization with reverse-transcribed ^{33}P-labeled cDNA probes.
(*Lower right*) Synthetic microarray data of the same images as used for intensity measurements.
Fig. 2 (*Upper left*) Synthetic microarray image based on the ratios of the intensities of expressed genes in the cisplatin-resistant cells (green spots) versus its parental cells (red spots). Green spots represent upregulated and red spots downregulated genes in cisplatin-resistant cells. Yellow spots depict equal gene intensities.
(*Upper right*) Scatterplot of correlation between transcript levels in the cisplatin-resistant cells and its parental cells. Solid lines indicate a difference by a factor of three. A considerable number of genes deviates more than 3-fold.
(*Middle left*) and (*Middle right*) Upregulated (green spots) and downregulated (red spots) groups of genes in cisplatin-resistant cells respectively. Using cluster analysis, functionally inter-related genes can be grouped together.
(*Lower left*) Semiquantitative RT-PCR validation of one upregulated gene, zinc finger protein 36 (Zfp-36), in cisplatin-resistant cells showing a consistent result.
(*Lower right*) Semiquantitative RT-PCR validation of one downregulated gene, cytochrome C oxidase subunit 15 (COX15), in cisplatin-resistant cells showing a consistent result.

References

1. Sgroi DC, Teng S, Robinson G, LeVangie R, Hudson JR, Elkahloun AG. In vivo gene expression profile analysis of human breast cancer progression. Cancer Res 1999; 59: 5656–5661.