Molecular profiling of human gliomas by cDNA expression array

Wei Zhang,[1] Alex Chenchik,[2] Stephen Chen,[2] Paul Siebert[2] and Chang Hun Rhee[1]

Extensive research in this decade has led to detailed understanding of genetic changes underlying human cancers. Two major tumorigenic events are mutation and amplification of oncogenes and inactivation of tumor suppressor genes. These events then trigger a series of signal transduction cascades, activating expression of downstream genes that control various cellular activities including cell cycle progression, DNA synthesis, programmed cell death, DNA repair, and cell migration. Investigations of these molecular pathways has led to the identification of many targets for therapeutic intervention. Knowledge of the expression patterns and functions of all human genes will provide a frame work for future molecular, genetic medicine. During the past ten years, the human genome project has generated an enormous amount of sequencing information, and sequencing of the entire human genome may be completed by the year 2003 (Aaronson *et al.*, 1996; Hiller *et al.*, 1996). One can envision that this will irreversibly transform the methodology of medical research and the practice of medicine. The search for new genes, which currently consumes the effort of many talented scientists, will become past history. Additionally, studying one gene at a time will be replaced by studying large number of genes simultaneously (Lockhart *et al.*, 1996). Reductionistic approaches to human disease will be replaced by systemic approach.

As a prelude to this revolution, tools used for parallel analysis of gene expression in the format of ordered gene arrays have been developed and are under continued expansion. In this technical tip, we will introduce the Atlas Human cDNA Expression Array system developed by Clontech Laboratories, Inc. (1997). With this technology, a conventional laboratory can profile the expression of 588 human genes simultaneously in one simple experiment without the using of expensive equipment. We will demonstrate the profiling of 588 genes in a human glioblastoma cell line to exemplify the utility of this technique.

The basic principle of Atlas Human cDNA expression Array can be viewed as a reverse northern blotting. cDNA fragments representing 588 human genes with known functions and known tight transcriptional controls are immobilized in duplicate onto a nylon membrane (Fig. 1). Each cDNA fragment is 200-500 bp long and selected as unique sequence without poly A tail, repetitive elements, or highly homologous sequences to minimize cross-hybridization and nonspecific bindings of cDNA probe. ^{32}P-labeled cDNA probes are generated by reverse transcription of each analyzed poly A+ RNA sample in the presence of [α-^{32}P]dATP. Each cDNA probe is then hybridized to an Atlas array. After a high-stringency wash, the hybridization pattern can be analyzed by autoradiography and/or quantified by phosphorimaging. Intensity of hybridization signals are linearly correlate with concentration of target mRNAs present at levels of 0.01%-3% of the total poly A+ RNA population. The background level (non-specific binding of ^{32}P-labeled cDNA probe to the membrane) is sufficiently low to permit detection of an mRNA that present at only 10-20 copies per cell - an abundance level of about 0.005% sensitivity. Because the amount of each cDNA fragment on the membrane is in excess (10 ng), binding of cDNAs to the probes is a linear event if RNAs are present at levels of 0.01%-3% of the total population.

Using this cDNA array technology, we profiled the gene expression pattern of the human glioblastoma cell line U251 before and after treatment with chemotherapeutic agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). ^{32}P-labeled cDNAs were synthesized and used to hybridize the cDNA array blot. After stringent wash and autoradiography, two expression profiles of 588 genes in U251 cells before and after BCNU treatment were obtained (Fig. 2). Comparison between the two blots identified seven genes whose expression was changed. Two of them increased and five of them decreased after BCNU treatment. Among the genes that showed decreased gene expression are platelet-derived growth factor receptor (PDGF-R) alpha and integrin beta 4. PDGF-R gene has been implicated in brain tumorigenesis

[1] Department of Neuro-Oncology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA
[2] Clontech Laboratories, Inc. 1020 East Meadow Circle, Palo Alto, CA 94303-4230, USA
Correspondence: Wei Zhang, Department of Neuro-Oncology, Box 316, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA, Tel: 713-792-3778, Fax: 713-745-1183

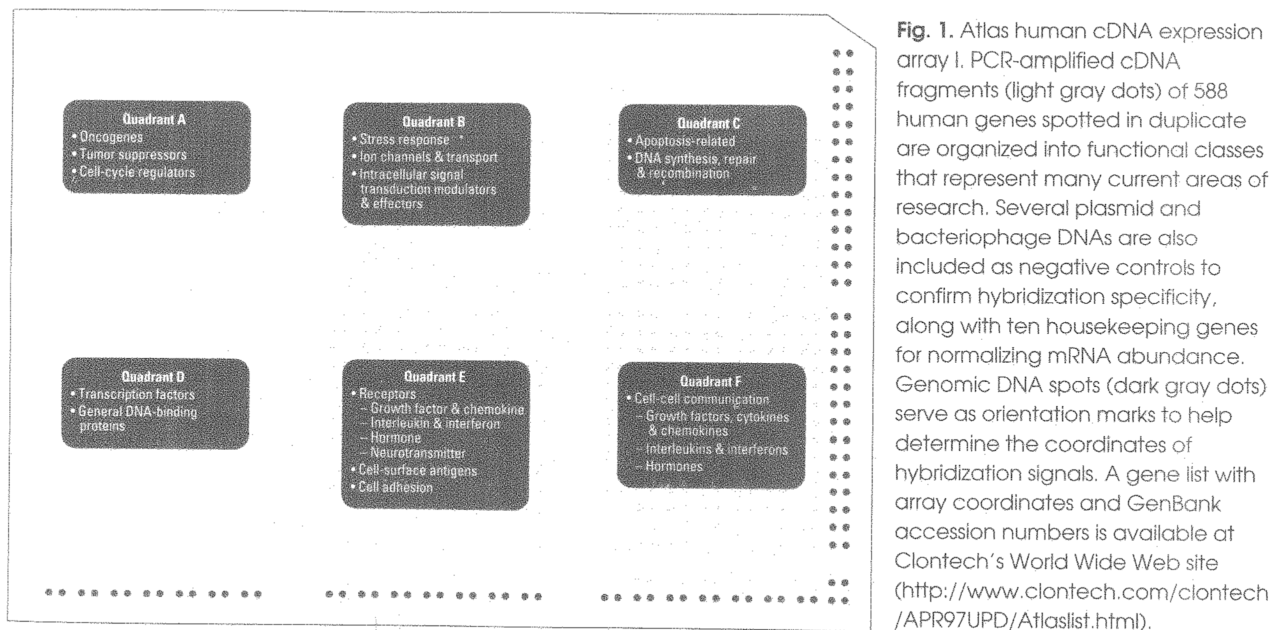

Fig. 1. Atlas human cDNA expression array I. PCR-amplified cDNA fragments (light gray dots) of 588 human genes spotted in duplicate are organized into functional classes that represent many current areas of research. Several plasmid and bacteriophage DNAs are also included as negative controls to confirm hybridization specificity, along with ten housekeeping genes for normalizing mRNA abundance. Genomic DNA spots (dark gray dots) serve as orientation marks to help determine the coordinates of hybridization signals. A gene list with array coordinates and GenBank accession numbers is available at Clontech's World Wide Web site (http://www.clontech.com/clontech/APR97UPD/Atlaslist.html).

(Westemark *et al.*, 1995), and integrin is known to be involved in tumor invasion (Tysnes *et al.*, 1996). Decrease in expression of these two gene reflects the beneficial effect of BCNU in brain tumor treatment. These results illustrated the utility of this technique in monitoring the effect on gene expression of different therapeutic modalities. This technology can also be used in identifying tissue specific, developmental-stage specific, and tumor specific gene expression patterns.

In addition to identification of differentially-expressed genes in cells before and after treatment of BCNU, the cDNA array reveals the most abundantly expressed mRNAs in U251 cells. Among these genes are cyclin B1, cyclin D1, a series of heat shock proteins, glutathione S-transferase, topo IIa, and Y-box DNA binding protein. Expression of these genes did not change by the treatment of U251 cells with BCNU. Profiling of additional glioblastoma cell lines and primary patient samples will reveal the genes that are most commonly expressed in this type of tumor.

The cDNA array technology can be applied to many areas of genetic medicine, and high-throughput gene expression

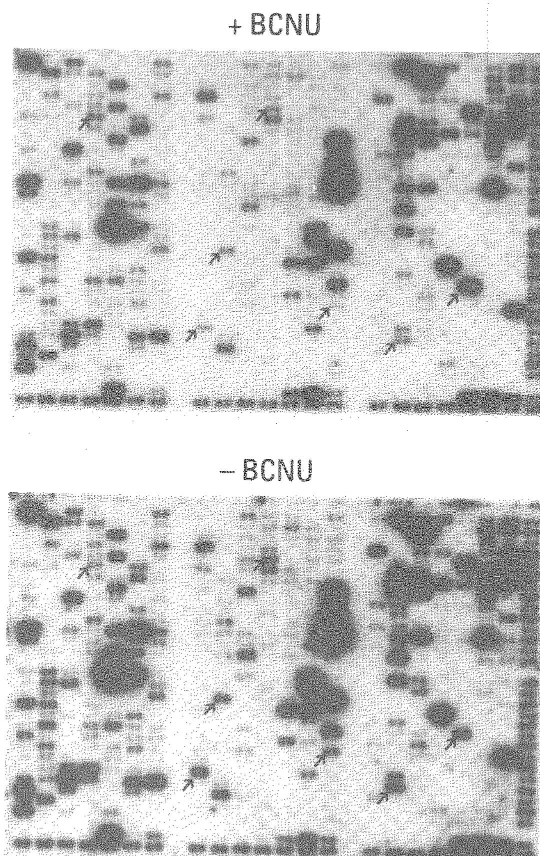

Fig. 2. Gene expression in human glioblastoma cell line U251 before and after BCNU treatment. Total RNAs of from the glioblastoma cells before and after BCNU treatment were isolated by TRI REAGENT (Molecular Research Center, Cincinnati, Ohio), and used for purification of poly A+ RNAs using mRNA isolation column (QIAGEN Inc., Chatsworth CA), using supplier's recommendations. Because minor genomic DNA contamination in the RNA may cause background signals, the mRNAs were treated with RNase-free DNase. Radiolabeled cDNAs were reverse transcribed from the poly A+ RNA and hybridized to the Atlas cDNA array (CLONTECH Laboratories, Inc., Palo Alto, CA) following the supplier's protocol. Blots were exposed to X-ray film for one day. Differences in gene expression on the blots are indicated by arrowheads.

analysis can be extended to a large cohort of patients. Gene expression profiling may provide a system for patient classification, for identification of key genes and pathways of genetic events, and for identification of potential targets for therapeutic intervention. Comparison of profiles in cells before and after therapeutic drug treatment may further identify the molecular mechanisms of drug action, sensitivity or resistance. Tremendous information has already been obtained from the first cDNA array technology. With the emergence of additional arrays in the coming year, we will no doubt contribute to an information explosion in biomedicine.

REFERENCES

Aaronson, J. S., Eckman, B., Belvins, R. A. *et al.* (1996) Toward the development of a gene index to the human genome: an assessment of the nature of high-throughput EST sequence data. *Genome Res* **6:** 829-845

Atlas Human cDNA Expression Array I (1997), *CLONTECHniques* **XII (2):** 4-7

Hiller, L. D., Lennon, G., Becker, M., Bonaldo, M. F., Chiapelli, B. *et al.* (1996) Generation and analysis of 280,000 human expressed sequence tags. *Genome Res* **6:** 807-828

Lockhart, D. J., Dong, H., Byrne M. C. *et al.* (1996) Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotechnol* **14:** 1675-1680

Tysnes, B. B., Larsen, L. F., Ness, G. O., *et al.* (1996) Stimulation of glioma-cell migration by laminin and inhibition by anti-alpha3 and anti-beta1 integrin antibodies. *Int J Cancer* **67:** 777-784

Westermark, B., Heldin, C. H. and Nister, M. (1995) Platelet-derived growth factor in human glioma. *Glia* **5:** 257-263