GENETICS

The Use of Large-Scale cDNA Analysis to Profile Differential Gene Expression in KYSE 410 Human Esophageal Cancer Cells after Irradiation

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Atlas Human cDNA Expression Array, which includes 588 genes divided into functional classes, was used to study gene expression profiles in KYSE 410 human esophageal cancer cells irradiated in doses of 6 and 18 Gy. Considerable changes in the expression of a number of genes was found, including down-regulation of 3 cell cycle regulators and up-regulation of an apoptosis-related gene. Comparison of the expression profiles and identification of genes were performed with Atlas Vision 3.0 software.

Key Words: *cDNA analysis; irradiation; transcription; carcinogenesis; genes; regulation of cell cycle; apoptosis*

Damage to DNA induces a variety and transcription events in cells, which limits the use of traditional RT-PCR. Large-scale cDNA analysis is now available, which allows easy and accurate profiling of gene expression [1,2]. Using the Atlas Human cDNA Expression Array I including 588 genes playing a key role in a variety of biological processes it is now possible to obtain more comprehensive information about genes involved in tumorigenesis.

Our objective was to profile irradiation-induced changes in gene expression in KYSE 410 human esophageal cancer cells using large-sale cDNA analysis.

MATERIALS AND METHODS

Human esophageal cancer cells (KYSE 410) were cultured in Eagle's modified minimal essential medium supplemented with 10% fetal bovine serum and antibiotics. The cells were irradiated in doses of 6 and 18 Gy, poly-A RNA was isolated 4 and 24 h postirradiation. The Atlas™ Human cDNA Expression Array I (Table 1) was used to characterize gene expression [1, 2]. ^{32}P-labeled cDNA was prepared from poly A$^+$RNA, hybridized on a nylon membrane with immobilized cDNA for different genes, each under strict transcriptional control (Fig. 1). Hybridization signals were normalized to that of housekeeping genes (Fig. 2).

Statistical sedimentation was used to determine differences in gene expression between groups. Atlas Vision 3.0 Software automated analysis was used to simplify the comparison of expression profiles [3].

RESULTS

Irradiation induced significant changes in the transcription of different genes, including down-regulation of 3 cell-cycle regulators and up-regulation of an apoptosis-related gene.

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Among oncogenes and tumor suppressors, high-abundance genes *myc*- proto-oncogen, transcription factors AP-1, FRA-, ezrin, EB1 showed reduced transcription.

Among the cell cycle control proteins, high-abundance genes p14-CDK inhibitor, protein kinase CLK (CLK3), and RACHI; medium-to-abundance genes galactosyl transferase-associated protein kinase, CDC2-related protein kinase, and cyclin D2 showed reduced transcription.

Among modulators, effectors, and intracellular transducers, the high-abundance genes guanine nucleotide regulatory protein NET1 and protein tyrosine kinase CAK; medium-to-low abundance genes stress-activated protein kinase JNK1, plasmascrine protease inhibitor, and receptor tyrosine kinase ligand LERK-4 showed reduced transcription.

Among apoptosis-associated proteins, high abundance genes NIP3, apopain, and HDLC1; medium-to-low abundance genes apoptosis inhibitor IAP, DADI, ICH-2 protease, and apoptosis cysteine protease MCH4 showed reduced expression.

Among proteins involved in DNA synthesis, repair, and recombination proteins, high abundance genes DNA-repair protein XRCC1, UV excision repair protein RAD23, GADD45, and DNA-dependent protein kinase (DNA-RK) showed reduced transcription.

Among DNA-binding and transcription proteins and transcription factors, high abundance genes erythroid krueppel-like transcriptional factor, 60S ribosomal protein L6, guanine nucleotide-binding protein G-S, transcriptional factor S-II-related protein, transcription factor LCR-F1; medium-to-low abundance genes CACCC box-binding protein, transcription factor ZFMI, RNA polymerase II elongation factor SIII, MtTF1, transcription initiation factor IIB, HTF4, annexin I showed reduced transcription; cell nucleic acid-binding protein and proliferation-associated PAG showed enhanced transcription.

In the cell receptor class, medium-to-low abundance gene interleukin-4 receptor α-chain showed reduced transcription.

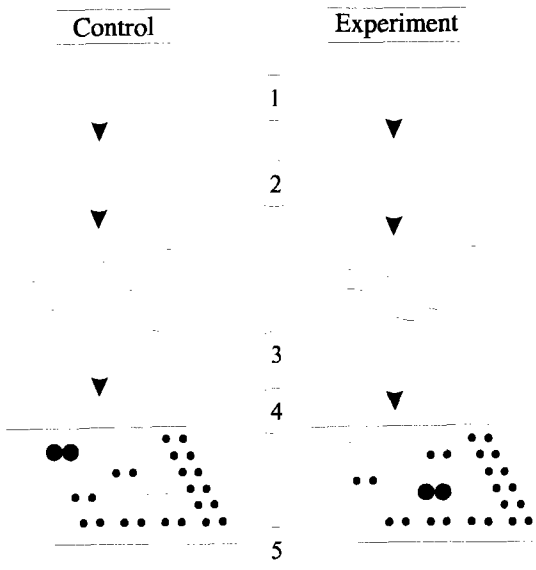

Fig. 1. Atlas procedure according to manufacturer's protocol with ^{32}P-labeled dATP. 1) isolation of polyA$^+$RNA; 2) preparation of complex cDNA probe; 3) hybridization and washout; 4) visualization of expression; 5) comparison of expression patterns.

Among cell surface antigens and adhesion proteins, high abundance genes vitronectin receptor-α, CD27L receptor, α-catenin, fibronektin receptor, integrin α_6, medium-to-low abundance genes CD19 B-cell antigen, B18 subunit, and integrin α_3 showed reduced transcription; integrin-αL showed enhanced transcription.

In the class of extracellular cell signaling and communication proteins, high abundance gene thymosin-β(10) showed reduced transcription and connective tissue growth factor showed reduced transcription.

Thus, large scale cDNA analysis is a powerful tool for studying expression of genes controlling complex cell phenotype. Expression patterns is important for identification of new therapeutic targets. Atlas Human cDNA Expression Array I was used to profile gene expression in U251 glioblastoma cell line before and after treatment with chemotherapeutic agent BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) [2,3]. It was found

TABLE 1. Atlas Human cDNA Expression Array I

Quadrant 1	**Quadrant 2**	**Quadrant 3**
Oncogenes	Stress-response	Apoptosis-related proteins
Tumor suppressors	Ion channels and transport	DNA synthesis, repair, and recombination
Cell-cycle regulators	Intracellular signal	
Transduction modulators and effectors		
Quadrant 4	**Quadrant 5**	**Quadrant 6**
Transcription factors	Receptors, growth factors, interleukins, hormones, neurotransmitters	Cell-cell communication, growth factors, cytokines and chemokines
General DNA binding proteins	Surface cell antigens	Interleukins, interferons
	Cell adhesion proteins	Hormones

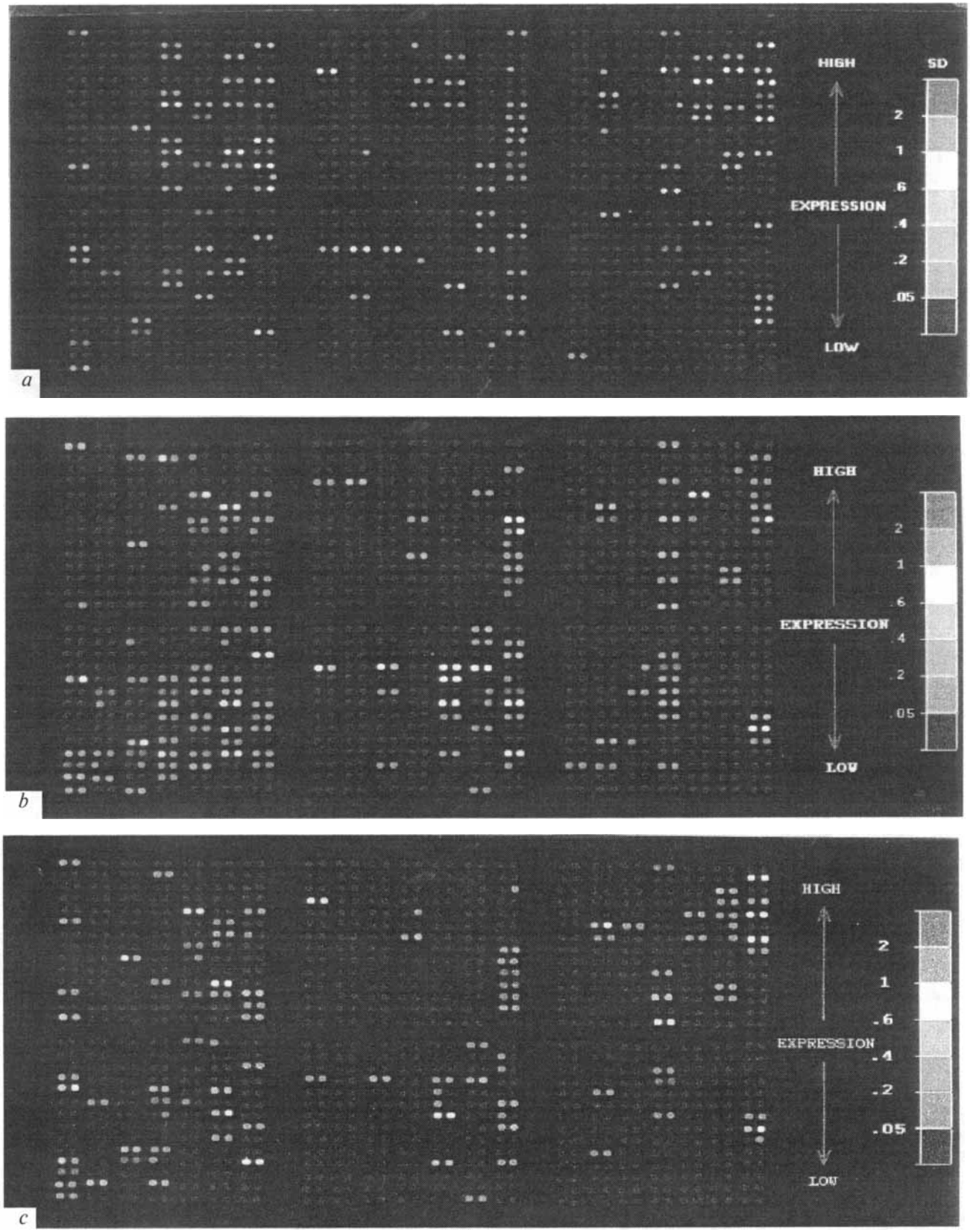

Fig. 2. Phosphorus image analysis using Atlas Vision 3.0 generating expression array color-coded according to signal intensity. *a*) nonirradiated control cells; *b*) irradiation in a dose of 6 Gy for 48 h, extraction after 24 h; *c*) irradiation in a dose of 18 Gy for 48 h, extraction after 4 h. Figures are printed in black and white here.

that platelet-derived growth factor receptor (PDGF-R) and integrin-4 are among the genes exhibiting reduced expression after BCNU treatment [2]. We found significant changes in gene transcription after irradiation of KYSE 410 human esophageal cancer cells, in particular, down-regulation of 3 cell cycle regulators and up-regulation of an apoptosis-related gene.

The wealth of sequence information necessitates more systemic approaches involving simultaneous and parallel analysis of hundreds and thousands genes. Analysis of tumor cells using Atlas array I and additional arrays that are now in production, will allow more objective analysis of the genes involved in tumorigenesis, their interrelations, biochemical pathways, and biological status.

REFERENCES

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