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Similarity of Gene Expression Profiles in Primary Brain Tumors with the Toxic Mechanism by Environmental Contaminants

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Abstract

Recently, a large number of clinical experiments have shown that exposure of organic pollutants lead to various cancers through the abnormal cell growth. Environmental pollutants, such as 2, 3, 7, 8-Tetrachloro dibenzo-*p*-dioxin (TCDD) and polycyclic aromatic hydrocarbons (PAHs), are carcinogen and are known to cause the cognitive disability and motor dysfunction in the developing of brain. The effects of these pollutants on neurodevelopmental disorder is well established, but the underlying mechanism(s) and similarity of gene expression profiles in human brain tumors with organic pollutants still remain unclear.
In this study, we first examined the gene expression profiles in glioblastomas compared with meningioma that are kinds of primary human brain tumor by using human cDNA microarray. The results of cDNA microarray analysis revealed that 26 genes were upregulated (Z-ratio > 2.0) and 14 genes were downregulated (Z-ratio < − 2.0) in glioblastoma compared with meningioma. From the altered gene patterns, mitogen-activated protein kinase (MAPK) signaling related genes, such as *MAP2K3, MAP3K11* and jun activated domain binding protein, and transcription factors, such as UTF2 and TF12, were upregulated in glioblastoma. Also, we tried to investigate the relation between important genes up- and down-regulated in giloblastoma and various organic pollutants. Therefore, the identification of changes in the patterns of gene expression may provide a better understanding of the molecular mechanisms involved in human primary brain tumors and of the relation between gene expression profiles and organic pollutants in brain tissue.

Keywords: glioblastoma, meningioma, organic pollutants, MAPK signaling, transcription factors, cDNA microrarry

In our environment, we are exposed numerous pollutants such as ultraviolet radiation (UV), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), heavy metal, and ozone. After pollutants are metabolized and accumulated in organism, these can become extremely toxic for the vital organs, after theses. Certain pollutants have a genotoxic effect, which can be immediate, delayed or even potentate each other[1]. Epidemiological studies have shown that environmental pollutants can cause many human cancers including lung cancer, skin cancer and hepatocellular carcinoma[1-4]. Especially, cancer in children may be result from prenatal exposure to pollutants that may alter germ cells or act as transplacental carcinogens.

Organic pollutant such as PAHs and TCDD accumulates in the brain, and then induce cognitive impairment and motor dysfunctions due to deficiency in the neurodevelopment process[5,6]. Many studies shown that several patients who are motor vehicle-related workers, electrical workers, chemical and petroleum industry's workers, and metal workers are associated with an increased risk of childhood brain tumors. Recent epidemiological study at Italy, France and Spain and population-based case-control studies at seven countries including Australia, Israel, France, Canada, Italy, Spain, and USA reported that parental occupation, occupational exposure to PAHs, a family of compounds released during incomplete combustion or pyrolysis of organic matters, might cause childhood brain tumors. Also, gestational exposure to TCDD, the prototype for a class of halogenated aromatic hydrocarbons, caused neurobehavioral and neurodevelopmental deficits in their off spring[7,8]. However, the effects of organic pollutants on the molecular alterations and genetic classification of glioblastoma and meningioma isn't still well defined.

Primary brain tumors are among the top 10 causes of cancer related death. The basic classification of

primary brain tumors by the World Health Organization (WHO) relies on their cellular origin, and describes five different groups: tumors originating from neuroepithelial cells, from cranial and spinal nerves, from meninges, from lymphatic and haematopoietic tissue, and from germ cells. Glioblastomas originate from the glial cells of brain tissue and are the most frequent type of primary brain tumors. Glioblatomas remain one of the most lethal forms of cancers with a median survival of 10 to 12 months[9,10]. Unlike other types of cancer, it induces death thought striking resistance to current therapies and invasion into normal brain tissue. Another common central nervous system neoplasm is meningiomas that comprise approximately 20% of all intracranial and 25% of all intraspinal neoplasm[11]. Although there are many alternative therapies for glioblastoma and meningioma, these have failed and limited to use radiation[12]. Therefore, new therapies to specific molecular targets are under development for two primary brain tumors, and advances in such approaches will require the roles of specific genes in it. In the present study, we investigated the gene expression profiles in glioblastoma, a human primary brain tumor, compared with glioblatoma, and we focused on the use of cDNA microarrays as a means of identifying similarity of gene expression profiles in human brain tumors with gene expression patterns affected by organic pollutants.

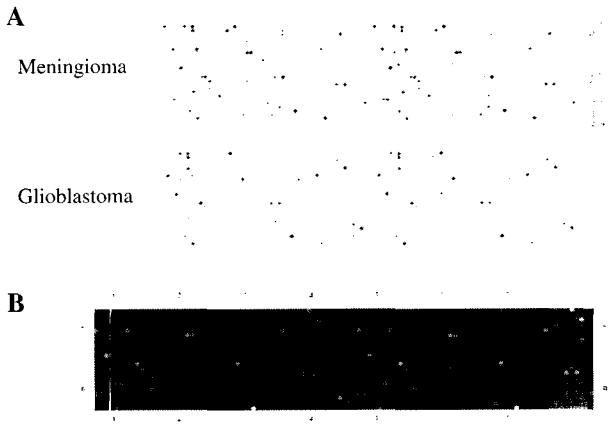

Fig. 1. Superimposed images of primary images of meningioma and glioblastoma (A) Representative cDNA microarrays of two independent hybridization experiments showing cDNA generated from meninigioma (up) or from glioblastoma (down). The cDNA microarray contained two sets of 2,304 genes and was printed in duplicate. Each duplicate was composed of eight individual subarrays. (B) Gene expression profiles of two human primary brain tumors. The figure shows duplicate grids for each gene spotted on the microarray membrane.

Superimposed Image of Primary Images of Two Human Primary Brain Tumors

To determine the difference of gene expression levels in glioblastoma and meningioma, we performed human cDNA microarray expression analysis. Radioactive hybridization was visualized by phosphoimaging. The primary image, which was result of the primary capture by using phosphoimaging is shown in Fig. 1. This particular array was printed in duplicate (as indicated by the line) and each duplicate was composed of six individual subarrays. Visual inspection of the hybridization patterns readily identified a number of signals differentially expressed in the glioblastoma and meningioma. Fig. 1 shows superimposed images in which the color red represents upregulation, green represents downregulation, and yellow represents the upregulation of genes.

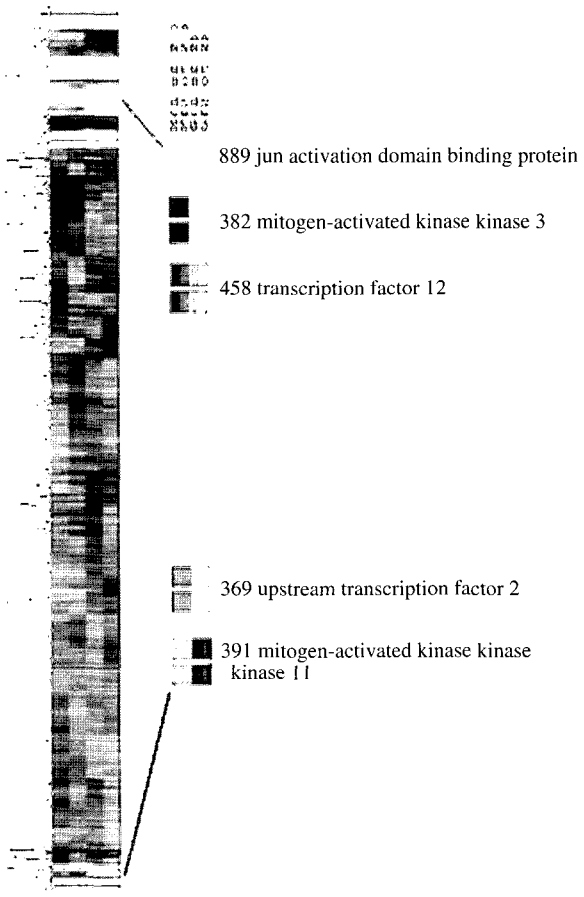

Fig. 2. Hierarchical clustering based on upregulated genes in primary human brain tumor. Cluster analysis of genes whose expressions changed in glioblastoma. A representative cluster diagram for genes related to MAPK singnaling and transcription factors. Upregulated genes are show in green and downregulated genes in red.

Table 1. Expression profiles of upregulated genes in glioblastoma, primary human brain tumor

Gene name	Z-ratio
Ubiquitin-conjugating enzyme E21 (homologous to yeast UBC9)	6.74
Insulin-like growth factor binding protein 2 (36kD)	6.20
Guanine nucleotide binding protein-like 1	5.23
Axonal transport of synaptic vesicles	4.79
Mitogen-activated protein kinase kinase 3	4.42
ADP-ribosylation factor-like 3	4.10
Chromobox homolog 3 (Drosophila HP1 gamma)	3.75
Upstream transcription factor 2, c-fos interacting	3.68
RAB interacting factor	3.67
Integrin-linked kinase	3.40
Homo sapiens clone 24703 beta-tubulin mRNA, complete cds	3.25
Regulator of G-protein signaling 13	3.24
Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase	3.11
Solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory doamin	3.04
Syntaxin 5A	2.80
profilin 1	2.60
Jun activation domain binding protein	2.59
Wingless-type MMTV integration site family member 2	2.49
Proteasome (prosome, macropain) subunit, alpha type 1	2.41
Transcription factor 12 (HTF4, helix-loop-helix transcription factor 4)	2.39
Rho GDP dissociation inhibitor (GDI) beta	2.34
Succinate dehydrogenase complex, subunit D, integral membrane protein	2.34
Mitogen-activated protein kinase kinase kinase 11	2.21
Serine threonine protein kinase	2.14
Proteasome (prosome, macropain) 26S subunit, non-ATPase, 10	2.02

Table 2. Expression profiles of downregulated genes in glioblastoma, primary human brain tumor

Gene name	Z-ratio
Tumor susceptibility gene 101	−6.37
Integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated	−5.41
Human DNA sequence from clone 366N23 on chromosome 6q27. Contains	−4.01
Platelet/endothelial cell adhesion molecule (CD31 antigen)	−3.44
Insulin-like growth factor 2 receptor	−3.38
S-antigen; retina and pineal gland (arrestin)	−3.27
Protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform	−3.20
Insulin-like growth factor 2 (somatomedin A)	−2.99
CD24 antigen (small cell lung carcinoma cluster 4 antigen)	−2.81
Chloride intracellular channel 1	−2.74
TYRO protein tyrosine kinase binding protein	−2.56
Inõsitol polyphosphate phosphatase-like 1	−2.53
Guanine nucleotide binding protein (G protein), alpha z polypeptide	−2.10
Synaptosomal-associated protein, 23kD	−2.01

Gene Expression Profiles Differences in Glioblastoma and Meningioma

All hybridization spots in images were quantified and normalized against the internal control. Global patterns of genes expression showed that 26 genes were upregulated (Z-ratio > 2.0) and 14 genes were downregulated (Z-ratio < −2.0) in glioblastoma compared with meningioma. The names and Z-scores of most prominently changed genes are listed (Table 1, 2). From the altered gene patterns, it is notable that mitogen-activated protein kinase (MAPK) signaling related genes were upregulated in glioblastoma. For example, mitogen-activated protein kinase kinase kinase 11 (*MAP3K11*), mitogen-activated protein kinas kinase 3 (*MAP2K3*), and Jun activated domain binding protein were upregulated (Table 1).

To obtain a molecular portrait of profiles of these genes in glioblastoma, we used a hierarchical clustering algorithm to group genes based on similar expression patterns. The data are presented in matrix format

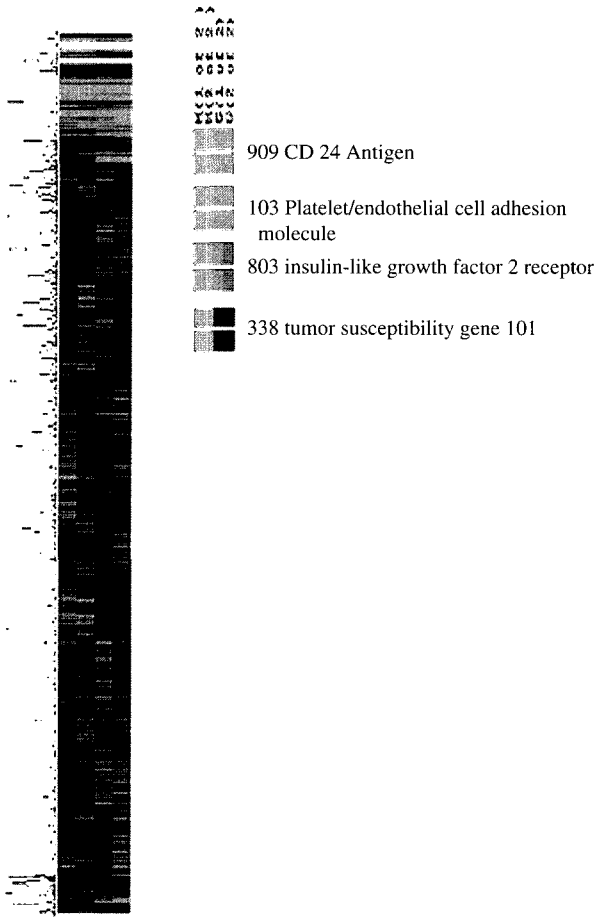

Fig. 3. Hierarchical clustering based on downregulated genes in primary brain tumor. Cluster analysis of genes whose expressions changed in glioblastoma. A representative cluster diagram of genes related to tumorogensis. Upregulated genes are show in green and downregulated genes in red.

(Fig. 2, and 3). Rows represent hybridization results for a single DNA element of the array, and columns represent the expression levels of all genes in a single hybridization sample. The expression levels of genes are represented by colors, and show expression relative to the median expression level of the particular gene across all samples. Red represents an expression greater than the mean and green represents expression less than the mean. Color intensity denotes the degree of deviation from the mean. Black represents the median expression level. Distinct samples representing similar gene pattern deviations from control cells are aligned in adjacent rows. Clustergrams revealed upregulated (Fig. 2 and Table 3) and downregulated (Fig. 3 and Table 1) clusters of genes in glioblastoma.

Discussion

Epidemiological studies have shown that the majority of human cancers are caused by environmental and life-style related factors. Cancer arises as result of accumulation of genetic changes that were induced by organic pollutants in genes of key molecules for cell growth and development. Many experimental evidences, which were conducted in US, Europe, Canada and Australia, shown that parental exposure to PAHs increased the risk of childhood brain tumors[7,8]. Although the effects of organic pollutants on development of brain tumors in childhood are well established, it remains unclear how primary human brain tumors in adulthood related to these organic pollutants.

To address this issue, we first examined the gene expression profiles in glioblastomas compared with meningioma that are kinds of primary human brain tumor by using human cDNA microarray. Also, we tried to investigate the relation between important genes up- and down-regulated in giloblastoma and various organic pollutants. In our study, cDNA microarray analysis revealed that 26 genes were upregulated (Z-ratio > 2.0) and 14 genes were downregulated (Z-ratio < -2.0) in glioblastoma compared with meningioma.

The results of the present study demonstrated that the expression of MAPK signaling related genes, such as *MAP2K3*, *MAP3K11* and Jun activation domain binding protein, was upreguated in glioblastoma. Constitutive expression of MAPKs is one of the important features of aberrant signaling leading to malignant transformation and tumor proliferation[13]. In the classic pathway, MAP3K and MAP2K, upstream regulators of MAPKs, phosphorylate and activate the MAPK, especially p38 kinase and extracellular signal-regulated kinase (ERK)[14-18]. Several investigators showed the relationship between these genes and TCDD. Wu *et al.* reported that activation of ERK enhances transformation and resistance to apoptosis in primary human glioblastoma cells[19]. In related to organic pollutant, Kim *et al.* reported that TCDD activated ERK-1/2 in cerebellar granule cells that might be contribute neurotocxicity in human[20]. Rummel *et al.* represented that PAHs stimulated ERK-type MAPK activity[21], also Lei *et al.* showed that benzo (a)pyrene induced MAPK and c-jun N-terminal kinase (JNK)[22].

In general, activated p38 and ERK by MAP2K phosphorylate transcription factors, including activated transcription factor 2 (ATF-2), Max and CCAAT enhancer-binding protein β (C/EBPβ), important in the regulation of cell growth. In particular, C/EBPβ

is known to play a crucial role in numerous cellular processes, such as cell growth and differentiation[23]. In addition, C/EBPβ is responsible for mediating cellular responses induced by environmental stressors[24]. Recently, several studies shown that TCDD induced the expression of C/EBPβ in many tissues including adipose tissue and embryonic fibroblast[25-27]. In our study, the expression of other transcription factors, such as upstream transcription factor 2 (UTF-2) and transcription factor 12 (TF12), and transcription regulator, jun activation domain binding protein, was upregulated in glioblastoma.

In summary, we identified a set of genes that were differentially expressed in glioblatoma, the primary human brain tumors. Furthermore, we explained the relevance between organic pollutants and gene expression profile in glioblastoma. In this study, we found that MAPK signaling related genes were over-expressed in glioblastoma as well as transcription factors, downstream effectors of MAPK. These genes are involved in a plethora of cellular processes that include cell proliferation, growth and development. Hence, the present study provides information useful for the understanding of the molecular mechanism(s) involved in human primary brain tumors and of the relation between gene expression profiles and organic pollutants in brain tissue.

Methods

Tumor Samples

All tissue samples were collected by the Catholic University of Korea. Eight samples of the 12 patients who underwent the surgical procedures were diagnosed with meningioma and rests of 4 samples were diagnosed with glioblastoma. Each tumor were frozen immediately after operation and stored at −70°C.

Human cDNA Microarray Construction

A human cDNA microarray was derived from a commercially available master set of approximately 15,000 human verified-sequences (Research Genetics, Inc.). The 15,000 human cDNA clone set was sorted for a list of genes (2,304 elements) representing gene families associated with differentiation, development, proliferation, transformation, cell cycle progression, immune response, transcription and translation factors, oncogenes, and with molecules involved in cell growth and maintenance. PCR-amplified cDNAs were spotted on nylon membranes.

Hybridization and Scanning

cDNA microarrays were pre-hybridized for 4hr at 42°C in 50 mL Falcon tubes in hybridization buffer containing 4.0 mL Microhyb (Research Genetics), 10 μL of 10 mg/mL human Cot 1 DNA (Life Technologies. Inc), and 10 μL of 8 mg/mL poly (dA) (Pharmacia, NJ) (both Cot 1 and poly (dA) were denatured at 95°C for 5 min prior to use). Approximately 10^7cpm/mL of heat-denatured (95°C, 5 min) probes were then added and incubated for 17hr at 42°C. Hybridized arrays were washed three times for 15 min in 2X SSC/0.1% SDS at room temperature and exposed to phosphorimager screens for 1-3days. The screens were then scanned in a Molecular Dynamics STORM Phosphorimager (Sunnyvale, CA) at a resolution of 50 μm.

RNA Preparation and cDNA Radiolabeling

Extraction of RNA from frozen tumor tissue was performed using Trizol Reagent followed manufacturer's instruction. Total RNA was used to synthesize ^{33}P-labeled cDNAs by reverse transcription. Briefly, 3 ~ 10 μg RNA was labeled using a reverse-transcription reaction in a reaction mixture of 40 μL containing 8 μL of 5X first strand PCR buffer, 1 μg of 1 μg/μL 24-mer poly (dT) primer, 4 μL of 20 mM dNTPs (minus dCTP), 4 μL of 0.1 M DTT, 40U of RNase, and 6 μL of 3000Ci/mmol α-^{33}P dCTP. The mixture was heated at 65°C for 5 min and incubated at 42°C for 3 min. Then, 2 μL (400U) of Superscript II reverse transcriptase (Life Technologies. Inc) was added, and incubated for 30 min at 42°C. Another 2 μL of Superscript II reverse transcriptase was then added and incubation continued for another 30 min at 42°C. Then, 5 μL of 0.5 M EDTA was added to chelate divalent cations and 10 μL of 0.1 M NaOH, and incubated at 65°C for 30 min to hydrolyze the remaining RNA. After adding 25 μL of 1 M Tris, pH 8.0, samples were purified using Bio-Rad 6 purification columns (Hercules, CA). This procedure resulted in an average of 5×10^6 to 3×10^7 cpm per labeled

Data Analysis

Array images were opened, cropped, and aligned using Image Quant Tools 2.1 (Amersham Pharmacia, Piscataway, NJ). Aligned images were then opened in Image Quant 5.1 and a pre-defining grid corresponding to the left side of the membrane was counted. The grid was then moved to the right side of the membrane, and duplicates were made. The data were normalized with Z scores by subtracting each average of gene intensity and dividing with each standard deviation. A Z score represents the variability from the average intensity, expressed in units of standard deviation, for each of the 2,304 genes. A Z score provides flexibility to compare different sets of microarray experiments by adjusting differences in hybridization

intensities. Gene expression differences between microarray experiments were calculated by comparing Z score differences for the same genes in different microarrays, which allowed gene expression to be compared in different samples. Z differences were calculated by subtracting the Z score of the control from each Z score of the control sample. These Z differences were further normalized to distribute their position by dividing with the average z difference and dividing with the standard deviation of the z differences. These distributions represented Z ratio values and allowed efficient comparisons to be made between microarray experiments. Scatter plots of intensity values were produced in Spotfire (Spotfire, Inc., Cambridge, MA). Color overlay images were produced in Adobe Photoshop 5.0 (Adobe Systems, Inc., San Jose, CA). Cluster analysis was performed on Z-transformed microarray data by using two programs available as shareware from Michael Eisen's laboratory (http://rana.lbl.gov). Clusterings of changes in gene expression were determined by using public domain Cluster based on pair wise complete-linkage cluster analysis. Raw gene expression, log values, and Z scores were averaged and are expressed as means ± standard deviation

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