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Microarray Analysis of Oxygen-Glucose-Deprivation Induced Gene Expression in Cultured Astrocytes

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Since astrocytes were shown to play a central role in maintaining neuronal viability both under normal conditions and during stress such as ischemia, studies of the astrocytic response to stress are essential to understand many types of brain pathology. The microarray system permitted screening of large numbers of genes in biological or pathological processes. Therefore, the gene expression patterns in the in vitro model of astrocytes following exposure to oxygen-glucose deprivation (OGD) were evaluated by using the microarray analysis. Primary astrocytic cultures were prepared from postnatal Swiss Webster mice. The cells were exposed to OGD for 4 hrs at 37°C prior to cell harvesting. From the cultured cells, we isolated mRNA, synthesized cDNA, converted to biotinylated cRNA and then reacted with GeneChips. The data were normalized and analyzed using dChip and GenMAPP tools. After 4 hrs exposure to OGD, 4 genes were increased more than 2 folds and 51 genes were decreased more than 2 folds compared with the control condition. The data suggest that the OGD has general suppressive effect on the gene expression with the exception of some genes which are related with ischemic cell death directly or indirectly. These genes are mainly involved in apoptotic and protein translation pathways and gap junction component. These results suggest that microarray analysis of gene expression may be useful for screening novel molecular mediators of astrocyte response to ischemic injury and making profound understanding of the cellular mechanisms as a whole. Such a screening technique should provide insights into the molecular basis of brain disorders and help to identify potential targets for therapy.

Key Words: Microarray, Gene expression, Astrocytes, OGD

INTRODUCTION

Astrocytes are the most numerous non-neuronal cell type in the central nervous system (CNS) and make up about 50% of human brain volume. Astrocytes perform several functions that are essential for normal neuronal activity, including glutamate uptake and release, K^+ and H^+ buffering, and water transport. Accordingly, astrocytes function can critically influence neuronal survival during ischemia and other brain insults. Astrocytes also influence neurite outgrowth and other processes that contribute to brain recovery in the post-injury period (Chen & Swanson, 2003). Effects of astrocyte on brain injury are divided into those with immediate influence on cell survival and those with long-term or delayed effects that influence later recovery and function.

In response to injury, astrocytes display characteristic phenotypic changes characterized as astrocytosis or gliosis. Astrocytes respond to damage of the nervous system by proliferating and adopting a reactive phenotype characterized morphologically by hypertrophic nuclei and cell bodies, and elaboration of distinct long and thick processes with increased content of glial fibrillary acidic protein (GFAP). In addition, reactive astrocytes express a wide variety of markers such as cytoskeleton proteins, cell surface and matrix molecules, proteases, protease inhibitors and several growth factors and cytokines (Ridet et al, 1997; Dong & Benveniste, 2001). By secreting diffusible factors, damaged neurons or activated astrocytes interact with immune cells and microglia in a complex manner.

In the setting of stress, such as during cerebral ischemia, dysfunction of astrocytes can severely disable the survival and function of surrounding neurons (Aschner, 2000; Kirchhoff et al, 2001). Although astrocytes are less vulnerable than neurons to an ischaemic insult (Goldberg & Choi, 1993; Mariff & Juurlink, 1999; Almeida et al, 2001; Reichert et al, 2001), their death greatly exacerbates the loss of brain homeostasis (Vernadakis, 1988; Bush et al, 1999; Sofroniew, 2000; Deitmer, 2001; Parish et al, 2002; Tanaka et al, 2002). Despite their central importance, the response of astrocyte to ischemic stress has not been extensively studied.

Understanding transcriptional changes in astrocytes after ischemia may provide strong basis to the researchers and therapeutic targets for treating stroke and promoting

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ABBREVIATIONS: OGD, oxygen-glucose deprivation; LDH, lactate dehydrogenase; CX, connexin.

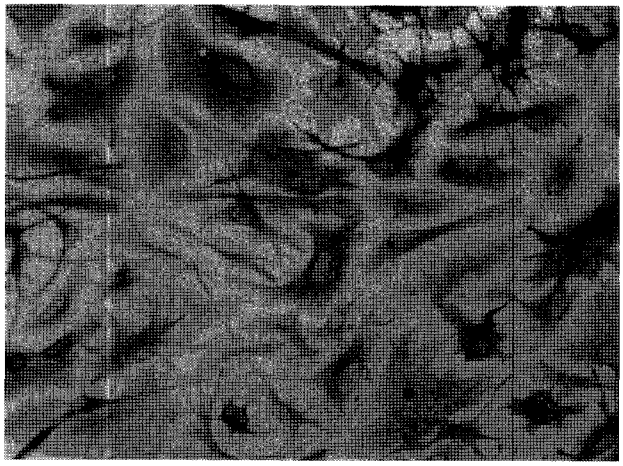

Fig. 1. Astrocytes are the major cell type of the whole cell population 5 days after plating. More than 95% of the cells show glial fibrillary acidic protein immunoreactivity (brown colored cells). 400× of magnification.

recovery to the clinicians. To study these changes in a genomic scale, we used murine astrocytic primary cultures in an in vitro model of ischemia, by subjecting them to combined oxygen and glucose deprivation (OGD) condition. In this study, the usefulness of the microarray analysis which allowed evaluation of large numbers of genes simultaneously in the model of in vitro ischemia was evaluated.

METHODS

Cell culture

Murine astrocyte cultures were prepared as previously described (Desahger et al, 1996). Briefly, newborn Swiss-Webster mice were anesthetized and then killed according to a protocol approved by the Kyungpook National University administrative Panel on Laboratory Animal Care, in accordance with the NIH guide. Brains were removed, freed of meninges, and the cortices minced and treated with 0.09% trypsin for 20 min at 37°C. After centrifugation at 400 g, cells were resuspended in plating medium containing Eagle's minimal essential medium (Gibco, Grand Island, NY, USA) supplemented with 10% equine serum (Hyclone, Logan, UT, USA), 10% fetal bovine serum (Hyclone, USA), 21 mmol/L glucose (Sigma, St Louis, MO, USA), 2 mmol/L glutamine (Gibco, USA), 26.8 mmol/L $NaHCO_3$ and 10 ng/ml epidermal growth factor (Sigma, St Louis, MO, USA) and triturated. The single cell suspension was plated in 24-well plates (Becton-Dickinson, Franklin Lakes, NJ, USA) at a density of 2 hemispheres/10 ml, or on 25 mm coverslips precoated with poly-D-lysine (Sigma, St Louis, MO, USA). Astrocyte cultures are more than 95% GFAP immunoreactive cells (Fig. 1), with the majority of remaining cells microglia, as identified by isolectin B4 staining. Cultures were subjected to injury after 5~7 days in culture before experiment.

Oxygen-glucose deprivation (OGD)

Cultures were transferred to an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, MI, USA) with an atmosphere of 5% CO_2, 5% H_2 and 90% N_2. The culture medium was replaced three times with deoxygenated, glucose-free balanced salt solution (BSS_0, pH 7.4) containing phenol red (10 mg/L) and the following (in mM): NaCl 116, $CaCl_2$ 1.8, $MgSO_4$ 0.8, KCl 5.4, NaH_2PO_4 1, $NaHCO_3$ 14.7, HEPES 10. $BSS_{5.5}$ uses as a control contains 5.5 mM glucose in BSS. Cultures were placed in a humidified 37°C incubator within the anaerobic chamber for 4 hrs. Oxygen tension was monitored with an oxygen electrode meter and was kept under 0.02%.

Cytotoxicity assay

Cytotoxicity of astrocytes was quantitated using enzyme assays-lactate dehydrogenase (LDH) activity in culture medium. LDH activity was carried out by a colorimetric method using a LDH assay kit (Sigma, St Louis, MO, USA).

RNA isolation, cDNA and cRNA synthesis

From the cultured samples, total RNA was isolated with RNeasy Midi kit (Qiagen, #75144, CA, USA) by the supplier's instructions. mRNA was purified from the total RNA samples (Qiagen, Oligotex Midi kit, #70042, CA, USA). Synthesis of cDNA and biotin-labeled cRNA, fragmentation, and hybridization were performed according to the Affymetrix Genechip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA, USA). Briefly, 20 μg of total RNA was used for cDNA synthesis using the Superscript II cDNA synthesis kit (Invitrogen, Superscript choice system for cDNA synthesis, #18090, USA); the cDNA was then cleaned by Phase Lock Gel Centrifugation (Eppendorf, Westbury, NY, USA). Biotin-labeled cRNA was synthesized by in vitro transcription of the cDNA (Ambio, Megascript T7 high yield transcription kit, #1334, USA) and then fragmented.

Microarray analysis

The fragmented cRNA was hybridized to duplicate Affymetrix Murine Genome U74v2, GeneChip A (Affymetrix, #900249, Santa Clara, CA, USA). The chip contains probe sets for 12,000 full-length mouse genes and EST clusters. Hybridization and scanning were done by the Stanford University core facility, with the Affymetrix Fluidic Station 400 and Gene Array scanner. Data acquisition was performed using the Affymetrix Microarray Suite version 4.0 and then the data were analyzed with dChip (Li & Wong, 2001; Available from http://biosun1.harvard.edu/complab/dchip/) and GenMAPP tools (Dahlquist et al, 2002; Available from http://www.genmapp.org/).

RESULTS

Cytotoxicity assay

OGD resulted in increased LDH release into the media in astrocyte cultures (Fig. 2).

Data processing

Array images are observed to check general signal intensities from the DNA chips after hybridization of the sam-

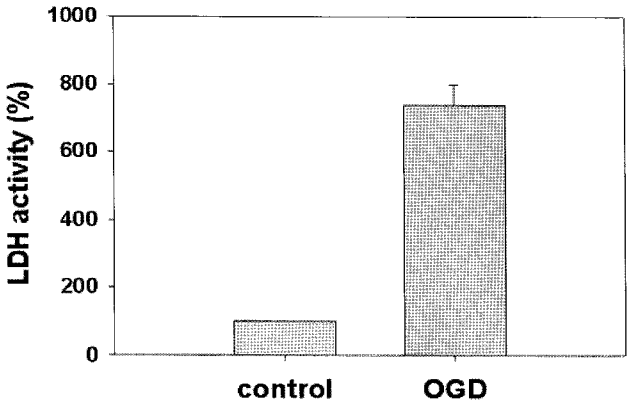

Fig. 2. Cell death of microglia was quantitated using enzyme assays-lactate dehydrogenase (LDH) activity in culture medium. LDH activity was carried out by a colorimetric method using a LDH assay kit. OGD resulted in increased LDH release in astrocytic cultures.

ples and probes on the chips (data not shown). After acquisition of the signals from the images, they were transformed into digital numbers and performed absolute analysis. To test the reproducibility of each microarray analysis, the absolute data from each chips (data not shown) were compared. Normalization and comparison of the data were made and hierarchical clustering was performed. The genes showing similar gene expression patterns are placed in the same group or clustered (Fig. 3).

Overall gene expression pattern during OGD

OGD exposure caused marked alterations of the expression levels of a large number of genes. To observe the global transcriptional activity after OGD exposure, we counted the numbers of genes showing altered transcriptional activities compared to control group at various criteria (Table 1).

When the threshold criteria were put at 1.5 fold, 131 genes passed the criteria. From 131 genes, 19 genes showed increased expression and 112 showed decreased expression level. The general decrease of the transcriptional activity after OGD exposure was consistent at all criteria we used. At 2 fold criteria, only 4 of 55 altered genes were increased genes. Thus we concluded that OGD exposure have general suppressive effect on the gene expression with the minor exceptions.

Functional classification of genes with altered expression during OGD

After the general estimation was done, we started to investigate the identities of individual genes. Even though the microarray analysis could provide enormous data, it is not easy to deal with all the genes. So we tried to reduce the number of genes by screening the genes with minimal transcriptional changes out and selected the genes which passed the criteria of 2 fold change. From the all genes over 2 fold changes (Table 2), we classified those genes into functionally related groups (Table 3). OGD suppressed expression of a large number of genes associated with immune, defense response, guanylate nucleotide binding proteins, chemokines, cytokine induced genes and histocompatibility proteins. The other genes, such as ATP-binding pro-

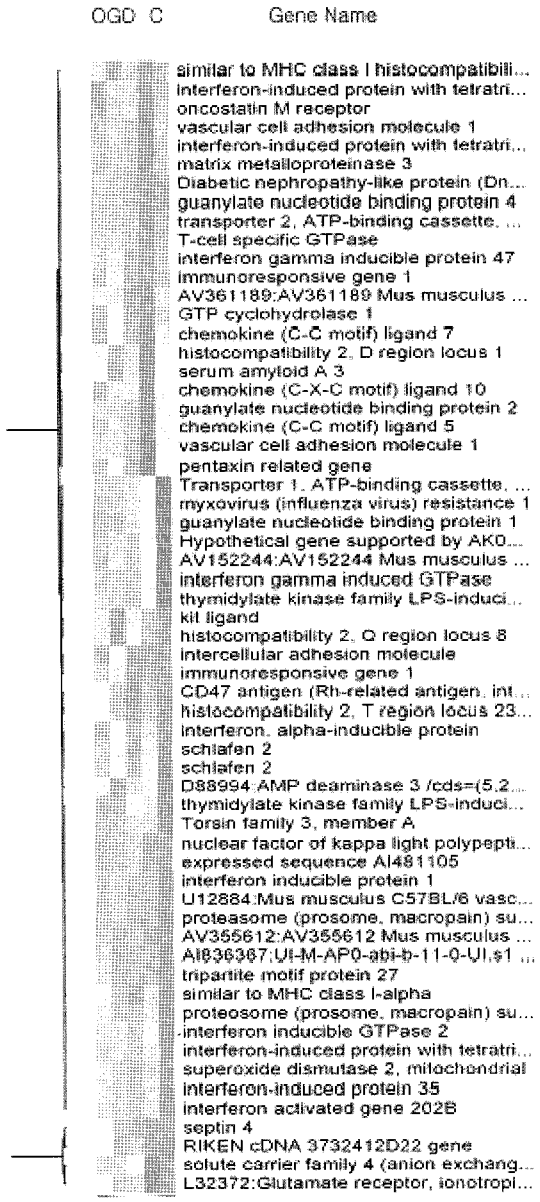

Fig. 3. Hierarchical clustering. Genes showing similar patterns are clustered when data from each experiments are compared with each other. Control conditions (C) were repeated twice and oxygen-glucose deprivation conditions (OGD) were three times. Data in the same experimental conditions show similar patterns.

Table 1. Number of genes showing increased or decreased expression after OGD exposure compared to control at various fold change in primary cultured astrocytes

Fold change	Number of increase	Number of decrease	Total number
1.5	19	112	131
2	4	51	55

Table 2. Genes whose expression were changed more than 2-fold after OGD exposure in primary cultured astrocytes

Gene name	Genebank number	Fold change
RIKEN cDNA 3732412D22 gene	AI847230	3.07
Septin 4	X61452	2.86
Solute carrier family 4 (anion exchanger), member 4	AF020195	2.56
Glutamate receptor, ionotropic, AMPA2 (alpha 2)	L32372	2.44
Interferon gamma inducible protein 47	M63630	−2.44
Oncostatin M receptor	AB015978	−2.56
Histocompatibility 2, Q region locus 1	M18837	−2.62
Interferon-induced protein 35	AW121732	−2.66
L46855:Ret finger protein	L46855	−2.67
TAP binding protein	AV361189	−2.68
Expressed sequence AI481105	AW121646	−2.7
AW047899:UI-M-BH1-aln-a-06-0-UI.s1 Mus musculus cDNA	AW047899	−2.71
Kit ligand	M57647	−2.73
CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	AI848868	−2.75
Superoxide dismutase 2, mitochondrial	L35528	−2.78
AV355612:AV355612 Mus musculus cDNA	AV355612	−2.81
Histocompatibility 2, Q region locus 8	D90146	−2.82
Thymidylate kinase family LPS-inducible member	L32973	−2.91
Torsin family 3, member A	AI508931	−2.92
Histocompatibility 2, D region /// histocompatibility 2, D region locus 1	X00246	−3.09
GTP cyclohydrolase 1	L09737	−3.15
Schlafen 2	AF099973	−3.17
Interferon-induced protein with tetratricopeptide repeats 2	U43085	−3.25
Proteasome (prosome, macropain) subunit, beta type 10	Y10875	−3.31
AMP deaminase 3	D88994	−3.37
Intercellular adhesion molecule	M90551	−3.51
TAP binding protein	AI836367	−3.87
AI326621:mn88h10.y1 Mus musculus cDNA	AI326621	−3.98
Serum amyloid A 3	X03505	−4.1
RIKEN cDNA C920025E04 gene /// histocompatibility 2, T region locus 23	Y00629	−4.13
Interferon inducible protein 1	U19119	−4.77
Similar to H2-Q10 protein (LOC381094), mRNA	M27134	−4.89
Schlafen 2	AF099973	−4.94
Guanylate nucleotide binding protein 1	M55544	−4.96
Transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	U60091	−5.13
Interferon, alpha-inducible protein	X56602	−5.32
Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	U60020	−5.38
Vascular cell adhesion molecule 1	M84487	−5.72
11 days pregnant adult female ovary and uterus cDNA	U43086	−5.8
Proteosome (prosome, macropain) subunit, beta type 8 (large multifunctional protease 7)	U22033	−6.1
Immunoresponsive gene 1	AI323667	−6.24
Expressed sequence AI481100	AJ007972	−6.26
Chemokine (C-X-C motif) ligand 10	M33266	−6.48
Interferon gamma induced GTPase	U53219	−6.58
Immunoresponsive gene 1	L38281	−6.72
Pentaxin related gene	X83601	−7.22
M21038:Mouse (strain CBA) interferon-induced mutant Mx1 protein pseudo gene mRNA	M21038	−7.55
Vascular cell adhesion molecule 1	U12884	−7.98
Guanylate nucleotide binding protein 2	AJ007970	−8.32
Thymidylate kinase family LPS-	AV246064	−8.47
AW047476:UI-M-BH1-all-g-04-0-UI.s1 Mus musculus cDNA	AW047476	−8.57
Chemokine (C-C motif) ligand 7	X70058	−8.6
M31418:Interferon activated gene	M31418	−8.83
Interferon, alpha-inducible protein	AV152244	−9.46
Diabetic nephropathy-related gene 1 mRNA, partial sequence	AA816121	−9.71
Matrix metalloproteinase 3	X66402	−11.55
Interferon-induced protein with tetratricopeptide repeats 1	U43084	−13.84
U12884:Mus musculus C57BL/6 vascular cell adhesion molecule-1 truncated form T-VCAM-1 (VCAM-1) mRNA	U12884	−13.96
Chemokine (C-C motif) ligand 5	AF065947	−14.22
T-cell specific GTPase	L38444	−23.47

teins, enzymes and cell adhesion molecules were also suppressed. From the genes whose expression were up-regulated more than 2 fold were one of the glutamate ionotropic receptor, α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid 2 (AMPA2, Genbank number X57498), solute carrier family 4, anion exchanger (Genbank number AF020195), septin 4 (Genbank number X61452) and RIKEN cDNA 3732412D22 gene (Genbank number BC075634).

Apoptotic pathways

Since OGD is one of the well known causes of cell death and apoptosis is known to play an important role in ischemic cell death of astrocytes (Wiessner et al, 2000), we made close observations on the changes in the apoptotic pathway (Fig. 4).

Among the pro-apoptotic genes, tumor necrosis factor (TNF) receptor-associated factor (TRAF), fibroblast-associated cell surface (Fas) ligand, and Poly (ADP-ribose) polymerase (PARP) showed increased gene expression, while the other pro-apoptotic factors, such as Fas, caspases, Fas-associated via death domain (FADD), BAX, BAK, BH3 interacting domain death agonist (BID), cytochrome c (Cyt c), p38, c-myc, receptor-interacting serine-threonine kinase (RIP), and apoptosis protease activating factor-1 (Apaf-1) demonstrated decreased or subtle changes in gene expression.

Expression of anti-apoptotic factors, BCL-X, BCL-2, inhibitor of apoptotic protein (IAP), transformed mouse 3T3 cell

Table 3. Functional classification of genes whose expression were changed more than 2-fold after OGD exposure in primary cultured astrocytes

Function	Gene	Fold change
Endoplasmic reticulum	Proteasome (prosome, macropain) subunit, beta type 8 (large multifunctional protease 7)	−6.1
	Proteasome (prosome, macropain) subunit, beta type 10	−3.31
	TAP binding protein	−3.87
	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	−5.38
	Transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	−5.13
Chemotaxis	Chemokine (C-C motif) ligand 5	−14.22
	Chemokine (C-C motif) ligand 7	−8.6
	Chemokine (C-X-C motif) ligand 10	−6.48
	Interferon, alpha-inducible protein	−5.32
Response to stimuli	Guanylate nucleotide binding protein 1	−4.96
	Guanylate nucleotide binding protein 2	−8.32
	Histocompatibility 2, D region // histocompatibility 2, D region locus 1	−3.09
	Histocompatibility 2, Q region locus 1	−2.62
	Histocompatibility 2, Q region locus 8	−2.82
	Intercellular adhesion molecule	−3.51
	Interferon gamma inducible protein 47	−2.44
	Interferon inducible protein 1	−4.77
	Interferon-induced protein 35	−2.66
	Interferon-induced protein with tetratricopeptide repeats 1	−13.84
	Interferon-induced protein with tetratricopeptide repeats 2	−3.25
	Pentaxin related gene	−7.22
	RIKEN cDNA C920025E04 gene /// histocompatibility 2, T region locus 23	−4.13
	Serum amyloid A 3	−4.1
	Glutamate receptor, ionotropic, AMPA2 (alpha 2)	+2.44

TAP, transporter associated with antigen processing; MDR, multidrug resistance; −, decrease; +, increase.

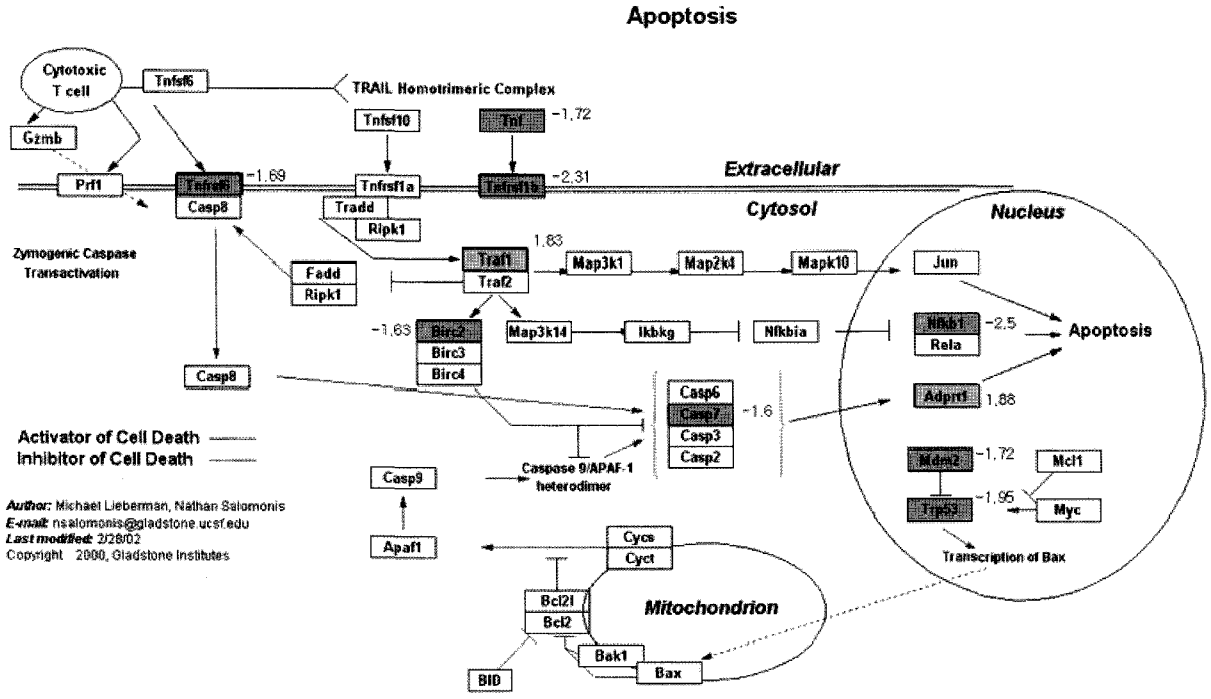

Fig. 4. Genes involved in apoptotic pathway following OGD in primary cultured astrocytes. Expression of the genes in pink is up-regulated and that of the genes in blue is down-regulated. Genes with more than 1.5 fold changes are presented.

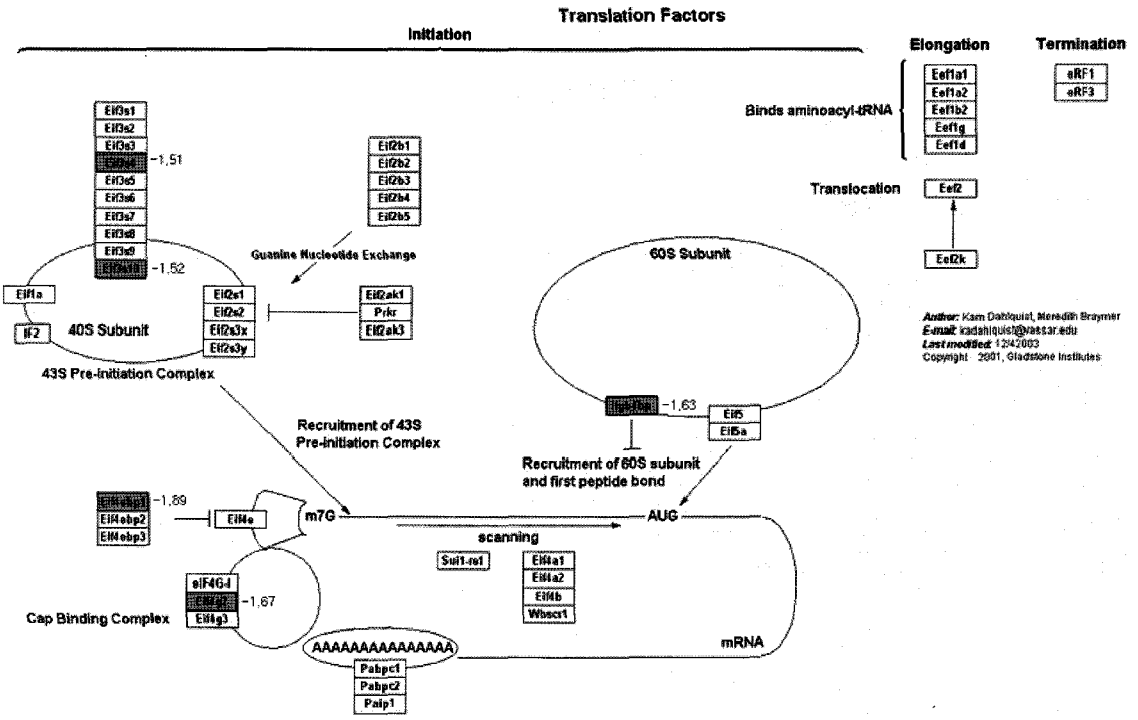

Fig. 5. Genes involved in protein translation following OGD in primary cultured astrocytes. Expression of the genes in pink is up-regulated and that of the genes in blue is down-regulated. Genes with more than 1.5 fold changes are presented.

double minute 2 (MDM2) and myeloid cell leukemia sequence 1 (MCL-1) were decreased or demonstrated no remarkable alteration following ischemic insult.

Gap junction proteins

It has been known that the intercellular communication is mediated by gap junctions composed primarily of connexin (Cx) 43, with minor contributions from the others. In this study the expression of connexin, component of gap junction channel, following OGD exposure were observed. Expression of Cx36 was increased by 3 folds and Cx43 by 1.5 folds while Cx57 was decreased. The other types of connexins were not changed significantly at gene expression level.

Protein translation factors

Most of the genes involved in protein translation, from initiation to termination steps, were down regulated by OGD (Fig. 5). eIF1A (eukaryotic translation initiation factor 1A) was the only gene with mildly increased gene expression. Other genes such as eukaryotic translation initiation factors (eIFs), eukaryotic translation initiation factor binding proteins (E-BPs), eukaryotic translation initiation factor 2 alpha kinases (eIF2αks), poly A binding protein (PAB), and eukaryotic translation elongation factors (eEFs) were decreased by OGD exposure.

DISCUSSION

In the present study, the gene expression patterns of primary cultured astrocytes following OGD exposure using microarray analysis were investigated. There have been no reports that applied microarray analysis technology to investigate the genome-wide responses of astrocyte to ischemic condition and this study is the first report in this field.

Astrocytes' responses after brain injury are divided into those with immediate influence on cell survival and those with long-term or delayed effects that influence later recovery and function. In this study, we focused on the immediate effect by limiting the experimental condition to 4 hrs of OGD exposure. It was revealed that OGD exposure, a strong detrimental stress to astrocyte, caused induction of very few genes and suppression of large number of genes. The number of genes whose expression were up-regulated more than 2 fold was only 4 while that of suppressed genes was 51. These suggest that OGD has general suppressive effect on gene expression with the exception of rare genes. We assume that exhaustion of energy lead to general suppression of the cellular mechanisms especially during or immediately after ischemic insult. Clear interpretation of the roles of increased genes is not possible at this moment and further investigation is required. However, we can speculate the meaning of some representative genes such as glutamate ionotropic receptor AMPA2, solute carrier family 4 anion exchanger or sodium bicarbonate cotransporter, septin 4 and RIKEN cDNA 3732412D22 gene which may be related with ischemic cell injury directly or indirectly.

Almost 50% of cells in CNS are astrocytes. They play an important role in normal physiological activity and have intimate relationship with neurons. There is a close bidirectional communication existing between neurons and astrocytes (Carmignoto, 2000). Glutamate, as the most important excitatory transmitter in CNS, is proved to be a crucial

bridge between astrocytes and neurons. Astrocytes responded to glutamate released from neurons by intracellular Ca^{2+} increase under physiological conditions (Shelton & McCarthy, 1999). In contrast to the physiological roles, glutamate induces excitotoxicity and thus contributes to neuronal degeneration in many acute CNS diseases, including ischemia, trauma, and epilepsy, and may also play a role in chronic diseases, such as amyotrophic lateral sclerosis.

There are three groups of ionotropic glutamate receptors, N-methyl-D-aspartic acid (NMDA), AMPA and kainic acid receptors. The other metabotropic glutamatergic receptors is coupled to G-proteins. NMDA receptor antagonists were excellent neuroprotective agents, but they produced psychotomimetic side effects, impaired learning and memory and induced morphological injury in cortical neurons in rats (Morris et al, 1986; Sonders et al, 1988; Olney et al, 1989). Thus, attention turned towards compounds, which block AMPA and kainate currents or selectively block AMPA currents (Parsons et al, 1998). Increase of glutamate ionotropic receptor AMPA2 may participate in the ischemic injury and it might be beneficial if the expression of this receptor can be blocked.

The electrogenic sodium bicarbonate cotransporter has been studied extensively in glial cells from different brain regions and from both invertebrates and vertebrates (Deitmer & Schlue, 1989; Newman, 1991; O'Connor et al, 1994). During physiological activation of neurons, a shift to acidic pH occurs (Trapp et al, 1996), and modulation of the extracellular pH by astrocytes may be critical to setting the level of excitability of neurons (Ransom, 1992). During neuronal activity, extracellular potassium increases, leading to glial depolarization and activation of the cotransporter (O'Connor et al, 1994). With activation of electrogenic sodium bicarbonate cotransporter the extracellular pH is acidified, while the intracellular pH of the astrocyte is alkalinized. The extracellular acidification may be instrumental in regulating local brain blood flow (Newman, 1991) in addition to its effect on neuronal excitability. This transporter plays an important role in physiological pH regulation and may play a critical role in pH regulation during pathophysiological events, such as brain ischemia (Lascola & Kraig, 1997). Cerebral ischemia is associated with a fall in intracellular and extracellular pH (Kraig et al, 1986), and more severe acidosis correlates with more severe injury (Myers & Yamaguchi, 1977).

Septins are a family of cytoplasmic proteins originally discovered in budding yeast (Saccharomyces cerevisiae) as essential proteins of cytokinesis (Hartwell et al, 1974). Recent studies suggest that yeast septins contribute to the cytoskeletal architecture by creating diffusion barriers within the dividing cytoplasm (Cooper & Kiehart, 1996; Frazier et al, 1998; Barral et al, 2000; Takizawa et al, 2000). In addition to this function, septins are related to many other important yeast division processes such as bud-site selection, chitin deposition, and spindle orientation (Faty et al, 2002; Longtine & Bi, 2003).

Septins have also been implicated in apoptosis. Apoptosis related septin (ARTS) is an alternative transcript of the SEPT4 gene that relocates to the nucleus on induction of apoptosis by transforming growth factor (TGF)-beta (Larisch et al, 2000). Studies have shown that overexpression of ARTS increases TGF-beta-induced apoptosis. Interestingly, as it happens with other apoptotic-regulating proteins such as Apaf-1 and CED-4 (Larisch et al, 2000). Recently, more insights about the apoptotic role of ARTS have been provided by Gottfried et al. (2004) and show that ARTS interacts with an inhibitors of apoptosis protein and induces a derepression of caspases to promote apoptosis.

Definite function of RIKEN cDNA 3732412D22 gene is not clear yet, it is known that this gene has homology with calcium binding proteins such as calponin or calmodulin.

Even though investigation of the role of specific gene per se is important, this can not provide overall understanding of the ischemic injury mechanism. To solve this difficulty, we made close observation of a couple of well-known mechanism pathways. This approach also has limitation in understanding the general phenomena but it can elucidate some specific pathways at least.

Since ischemia or OGD is one of the well known causes of cell death and apoptosis plays an important role in ischemic cell death of astrocytes (Wiessner et al, 2000), we observed the changes of the apoptotic pathway following OGD. Apoptosis is a form of programmed cell death that involves complicated cascades of related factors. Typical features of apoptotic cell death have already been found after transient ischemia, including degradation of DNA resulting in the typical ladder pattern on electrophoresis gels, cytochrome c release from mitochondria, and released cytochrome c-triggered activation of caspases. The data showed that some of the pro-apoptotic genes are increased while the others are decreased or not changed. Anti-apoptotic genes were decreased or not changed following ischemic insult. In contrast to my assumption, not all of the apoptotic cascades are activated but anti-apoptotic pathways seem to be suppressed.

We observed increase of connexin 36 and 43, and decrease of connexin 57 while other types of connexins were not changed significantly. Astrocytes regulate the composition of the extracellular environment and respond to the activity of adjacent glial and neuronal cells. One important characteristic of astrocytes is their electric and metabolic coupling through gap junctions. Gap junctions are widely expressed in various cell types of the nervous system and have been described to facilitate direct communication between astrocytes (Kielian & Esen, 2004). Gap junctions and their constituent connexin proteins have represented an investigational challenge in all tissues where they occur, but no structure is more complicated or more interconnected than the mammalian nervous systems. In the mammalian nervous system, at least six connexins (Cx26, Cx29, Cx30, Cx32, Cx36 & Cx43) have been identified at ultrastructurally defined gap junctions in neurons and glia (Rozental & de Carvalho, 2000), and at least five more (Cx31, Cx37, Cx45, Cx47 & Cx57) have been reported to be present in neural tissue (Nagy et al, 2004). Coupling by gap junction is thought to be at the core of numerous functions sustained by astrocytes ranging from detoxication of the extracellular spaces to participation to neurotransmission.

In accordance with this view, numerous agents have been found to modulate astrocyte gap junction communication. Interleukin-1 and other gap junction communication modulating factors are known to be upregulated following brain injury (Chen & Swanson, 2003). In some models of ischemia/anoxia, astrocytic gap junctions remain partly open and may contribute to the propagation of a death signal, and therefore to the size of the infarct. There are conflicting data regarding the effects of astrocyte gap junction coupling on ischemic injury.

An additional consideration is that some gap junction "hemichannels" open to the extracellular space rather than

to coupled cells. Recent studies suggest that these hemichannels may contribute to cell death and be regulated differently than coupled gap junctions.

Most of the genes involved in protein translation were down regulated by OGD, suggesting translational shutdown in astrocytes. Shutdown of translation is a common response of cells to a severe form of stress. This stress response is highly conserved from yeast to mammalian cells. The signal transduction cascade resulting in stress-induced shutdown of translation is initiated by activation of one of the kinases that specifically phosphorylates the alpha subunit of the initiation factor 2 (eIF2). Phosphorylation of eIF2 leads to eIF2 becoming an inhibitor of eIF2B. The consequent reduction in levels of active eIF2-GTP results in impairment of the initiation process of protein synthesis. Shutdown of translation triggered by overactivation of a eIF2 kinase or mutation of eIF2 (S51D), which mimics phosphorylated eIF2, is sufficient to cause cell death. One way to elucidate the significance of an ischemia-induced shutdown of translation for the affected cells, and to establish whether it contributes to cell death or protects cells from injury, is to focus on the underlying mechanisms and to understand the role of this process.

There is strong evidence that endoplasmic reticulum (ER) dysfunction is involved in the suppression of protein synthesis induced by transient cerebral ischemia. This study shows that some of the ER genes are significantly downregulated and ER dysfunction following ischemia may be partially related with this phenomenon. We now need to know why cells exposed to conditions associated with ER dysfunction respond to this pathologic process with a shutdown of translation and whether cells need to react in such a way as to withstand periods of impaired ER function.

In this study, we tried to verify that microarray analysis is a new useful tool to characterize biologic events in a in vitro model of ischemic injury. The evidences made at our hand demonstrated that OGD-induced injury of astrocytes was characterized by general suppression of gene expression with the exception of some cell death related genes. These results are consistent with previous reports (Chen & Swanson, 2003). In addition, we could understand the mechanisms more precisely. Here we suggest that microarray analysis of gene expression may be useful to elucidate novel molecular mediators of ischemic injury and making profound understanding of the cellular mechanisms as a whole. Such a screening technique might provide further insights into the molecular basis of brain disorders and identify potential targets for therapy.

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