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The Gene Expression Profiling in Murine Cortical Cells Undergoing Programmed Cell Death (PCD) Induced by Serum Deprivation

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PCD (programmed cell death) is important mechanism for development, homeostasis and disease. To analyze the gene expression pattern in brain cells undergoing PCD in response to serum deprivation, we analyzed the cDNA microarray consisting of 2,300 genes and 7 housekeeping genes of cortical cells derived from mouse embryonic brain. Cortical cells were induced apoptosis by serum deprivation for 8 hours. We identified 69 up-regulated genes and 21 down-regulated genes in apoptotic cells. Based on the cDNA microarray data four genes were selected and analyzed by RT-PCR and northern blotting. To characterize the role of UNC-51-like kinase (ULK2) gene in PCD, we investigated cell death effect by ULK2. And we examined expression of several genes that related with PCD. Especially GAPDH was increased by ULK2. Theses findings indicated that ULK2 is involved in apoptosis through p53 pathway.

Keywords: Apoptosis, Cortical cells, cDNA microarray, Screening, UNC-51-like kinase (ULK) 2

Introduction

Programmed cell death (PCD) is an important mechanism in both development and homeostasis in adult tissues for the removal of either superfluous, infected, transformed or damaged cells by activation of an intrinsic suicide program. Especially in brain programmed cell death is the essential component of neuronal development associated with many forms of neurodegeneration (Pettmann *et al*., 1998). According to previous study, programmed cell death divided two major types. Type I cell death is apoptosis and type II is autophagical cell death. These two cell death types are different from morphological criteria and molecular level. In morphological criteria, the character of type I cell death, apoptosis is cell shrinkage, nuclear condensation, nucleus DNA fragmentation without major ultra structural changes of cytoplasmic organelles and formation of apoptotic bodies. However in autophagical cell death double or multiple membranes cytoplasmic vesicles are appeared. These vesicles, autophagosome, engulf bulk cytoplasm and cytoplasmic organelles and are destroyed by the lysosome (Gozuacik *et al*., 2004). In molecular level, apoptosis is involved in caspase cascade through death receptor or mitochondrial alteration. In autophagical cell death, mTOR pathway is important pathway according to resent reports (Meijer *et al*., 2004). Especially, apoptosis is involved in broad range biological phenomenon including development, homeostasis and many diseases (Meier *et al*., 2000). Autophagical cell death is known that this process involved in neurodegeneration, retinal degeneration (Shintani *et al*., 2004) and bacterial infection (Nakagawa *et al*., 2004). However in present, many studies suggest that apotosis and autophagical cell death are interconnected in some condition; such as TNF-α-induced apoptosis (Jia *et al*., 1997) or amino acid deprivation (Martinet *et al*., 2005).

To find the PCD related pathway, we identified the novel components of the programmed cell death pathway. The cDNA microarray is used to screen and to compare the gene expression on a large scale. It is a useful technology that allows monitoring the expression level of numerous genes simultaneously (Yi *et al*., 2005). The goal of the present study is to screen genes related to PCD using the cDNA microarray and to study the role of the candidate gene UNC-51-like kinase 2 (ULK 2) in the neuronal PCD induced by the serum deprivation.

Materials and Methods

Cortical cell culture and the serum deprivation. Primary cortical

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cells were obtained from embryonic day 14 ICR mice. Cerebral hemisphere was mechanically dissociated and resulting cell suspension was seeded into poly-D-lysine and laminine coated culture flasks in Minimum Essential Medium (MEM). After primary cell culture for 6-7 days, serum deprivation was performed by washing each well twice with 750 μl of MEM containing 20 mM glucose and subsequent incubation in MEM containing 20 mM glucose.

The cDNA microarray hybridization and the data analysis. We used home made microarray. A microarray harboring the 2,304 unique mouse cDNAs was used that including 202 positive controls. We applied GAPDH and β-actin as a positive control. The Total RNA from neuronal cells was prepared using Trizol reagent following the manufacture's instruction (Invitrogen). 100 μg of total RNA obtained from neuronal cells were separately reverse transcribed into cDNAs in the presence of either Cy-3 or Cy5 dye - conjugated dUTP using superscript II reverse transcriptase. The labeled mixture thus prepared was applied to the microarray. After incubation for 16 h at 55°C, the microarray slides were washed in 1 × SSC/0.1% SDS for 2 min, followed by washing in 0.1 × SSC, 0.1 × SSC, and 0.1 × SSC for 10 min for three times with gentle agitation, and air-dried by spinning at a low speed. The microarray slides were scanned for Cy3 and Cy5 fluorescence using an ArrayWox reader (ArrayWox). ImGene^{TM} version 4.2 software (Biodiscovery) was used for the quantitation.

Northern blot analysis. Northern blot hybridization was performed with probes using the four PCR products - ULK2, HDAC2, MAGOH, and Sup. Ty4. The following primer pairs were used: ULK-2 sense 5'-ACAGACACCTTACGCCATCTGA-3'; ULK2 antisense 5'-CTCAGCTTCCCAGACTTGACCT-3'; HDAC2 sense 5'-AAGGAGGTCGTAGGAATGTTGCTG-3'; HDAC2 antisense 5'-ATCCCAGAATCGTCTCACTTTTCG-3'; MAGOH sense 5'-TAAATTGCGATACGCCAACAAC-3'; MAGOH antisense 5'-GG ATCCTTGGACTGGTTGACAT-3'; Sup. of Ty 4 homolog sense 5'-CTGGATTTGTAGGCCACTCCTC-3'; Sup. of Ty 4 homolog antisense 5'-TGTTTGCTGTGCTCGTTAGTCA-3'. PCR products were purified by gel extraction kit (D4001, Zymo research). Then 25-50 ng purified PCR product was labeled with [α-^{32}P]dCTP, using Random Primed DNA Labeling Kit (Roche). We made blots from primary cultured cortical cells and serum deprivated cortical cells. The blots was pre-hybridized with ExpressHyb solution (BD Clontech, USA) for 30 min at 60°C, then hybridized with probe in fresh ExpressHyb solution for overnight at 60°C. The blots were then washed several times before exposure to X-ray film at −70°C for 3 days.

Semi-quantitative RT PCR. The semi-quantitative analysis for the confirmation of cDNA microarray data was performed using the RT PCR method. The total RNA was extracted using Trizol reagent and the first strand cDNA was synthesized using superscript II reverse transcriptase (Invitrogen Life technologies). cDNA thus obtained was used as the template for the PCR amplification to generate the products corresponding to the mRNA encoding the gene product of interest. The PCR product was separated by the 1% agarose gel electrophoresis. GAPDH was used as the internal control.

Transient transfection. ULK2 gene obtained from I.M.A.G.E consortium (IMAGE ID 5709559) was cloned into pFLAG-CMV plasmid (Sigma) using two restriction endonuclease enzymes (*Not*I and *Sal*I). Mouse neuroblastoma cell line, BC3H1 cells were obtained from Korean Cell Line Bank. Cells were transfected with 500 ng of DNA by using the Lipofectamin 2000 reagent (Invitrogen).

MTT assay. BC3H1 cells were plated at the density 10^4 cells per well into 24 well plates. At the end of the gene expression for 48 h, 100 μl of MTT solution was added, and cells were incubated for 4 h. Colorimetric analysis was performed using ELISA reader with the 570 nm filter.

Annexin assay. ULK2 transfected Cells (1×10^5) were incubated for 48 h and then harvested. Specific binding of annexin V-FITC was performed by the incubation of cells for 15 min at room temperature in a binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4) containing a saturating concentration of annexin V-fluorescein isothiocyanate (FITC) and 7-amino-actinomycin D (7-AAD). After incubation, the cells were pelleted and analyzed in FACScan analyzer (Becton Dickinson). Annexin V^+/7-AAD^+ cells were defined as necrotic (or late apoptotic), while annexin V^+/ 7-AAD^- cells were defined as apoptotic.

Results

The profile of the gene expression induced by the serum deprivation profile of murine cortical cells. Here, we used the cortical neuronal cells to identify the genes inducing apoptosis in response to the serum deprivation. The cDNA microarray hybridization analysis was performed using 2,300 murine genes. To monitor the view of the overall gene expression in cortical cells following the serum deprivation, 8 h after the serum deprivation, genes that showed the difference by greater than 1.7 folds were selected. Applying the criteria, we identified 90 genes differentially expressed in cortical cells. Among them, 69 genes were up regulated and 21 genes were down regulated. (Table 1 and Table 2) We selected four genes among 69 up-regulated genes by Gene ontology analysis. The Gene Bank ID, the gene description, and the ratio are shown in Table 3. In these four genes, especially the expression level of ULK2 and HDAC2 were high. The examination of the original image data revealed that the ratio was not effect by the background (data not shown).

The confirmation of the differential gene expression by RT-PCR. To confirm the differential expression of the selected four genes described in Table 3, the semi-quantitative RT-PCR analysis and northern blot analysis were performed. Although the numerical value of the ratios of controls and the apoptotic neuron cells was different the RNA expression pattern is similar to the microarray data, (Fig. 1).

The functional study of ULK2. Among the four genes, the expression of this gene is very high in apoptosis by serum

Table 1. Up regulated genes list

Gene Bank ID	Gene Name	Fold Change
Mm.27291	Mus musculus bystin mRNA, complete cds	1.7
Mm.17484	synuclein, alpha	1.8
Mm.10808	Mus musculus RW1 protein mRNA, complete cds	1.8
Mm.4641	trefoil factor 3, intestinal	1.8
Mm.41755	death-associated kinase 2	1.8
Mm.3544	calcium channel beta 3 subunit	1.9
Mm.22584	serine/threonine kinase receptor associated protein	1.9
Mm.2038	ras-GTPase-activating protein SH3-domain binding protein	1.9
Mm.396	ubiquitin specific protease 9, X chromosome	1.9
Mm.41374	Trk-fused gene	1.9
Mm.6562	Mus musculus sulfotransferase-related protein (SULT-X1) mRNA	1.9
Mm.9002	ubiquitin conjugating enzyme E3A	2.0
Mm.28118	Fas death domain-associated protein	2.0
Mm.3759	max binding protein	2.0
Mm.10225	periplakin	2.0
Mm.2395	male enhanced antigen 1	2.0
Mm.3632	MAD homolog 4 (Drosophila)	2.0
Mm.26166	Mus musculus mRNA for protein-tyrosine-phosphatase IF1	2.1
Mm.16766	protein kinase, cAMP dependent, catalytic, beta	2.1
Mm.34537	CCAAT/enhancer binding protein (C/EBP), alpha	2.1
Mm.21772	Mouse beta-D-galactosidase fusion protein mRNA, complete cds	2.1
Mm.4493	Mus musculus endophilin I mRNA, complete cds	2.1
Mm.3288	regulatory protein, T lymphocyte 1	2.1
Mm.417	zinc finger protein 148	2.2
Mm.21904	Mus musculus partial mRNA for hypothetical protein (ORF37 DNA)	2.2
Mm.34778	fatso	2.2
Mm.29198	peroxisomal farnesylated protein	2.2
Mm.622	**suppressor of Ty 4 homolog (S. cerevisiae)**	**2.2**
Mm.34637	catenin alpha 2	2.2
Mm.2418	zinc finger protein 144	2.2
Mm.27657	Mus musculus C184L-22 mRNA, complete cds	2.2
Mm.29976	peanut-like 1 homolog (Drosophila)	2.3
Mm.16773	Aldehyde dehydrogenase 1 (phenobarbitol inducible)	2.3
Mm.2291	cytotoxic granule-associated RNA-binding protein 1	2.4
Mm.28746	Mus musculus YGR163w mRNA homologue, complete cds	2.4
Mm.3679	tetratricopeptide repeat domain	2.4
Mm.27664	**Mus musculus mRNA for UNC-51-like kinase (ULK) 2, complete cds**	**2.4**
Mm.36174	Mus musculus UDP-Gal:betaGlcNAc beta 1,3-galactosyltranferase-III	2.5
Mm.12951	Mus musculus type 6 nucleoside diphosphate kinase NM23-M6 (Nm23-M6)	2.5
Mm.1685	plexin 3	2.5
Mm.18502	Mus musculus mRNA for oxysterol-binding protein, complete cds	2.5
Mm.1383	guanosine diphosphate (GDP) dissociation inhibitor 5, Rho associated	2.6
Mm.3634	gene rich cluster, C3f gene	2.6
Mm.2591	RNA binding motif protein 3	2.6
Mm.2795	surfeit gene 4	2.6
Mm.13162	Mus musculus ERG-associated protein ESET mRNA, complete cds	2.6
Mm.4606	branched chain aminotransferase 1, cytosolic	2.7
Mm.831	signal sequence receptor, delta	2.7
Mm.22665	histone deacetylase 5	2.7
Mm.19806	**histone deacetylase 2**	**2.7**
Mm.2849	heat shock protein, 74 kDa, A	2.7

Table 1. Continued

Gene Bank ID	Gene Name	Fold Change
Mm.8884	nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	2.8
Mm.2386	secretogranin III	2.9
Mm.43660	palmitoyl-protein thioesterase	3.0
Mm.17224	speckle-type POZ protein	3.0
Mm.1540	Mus musculus eIF-1A (eIF-1A) mRNA, complete cds	3.0
Mm.10508	Sjogren syndrome antigen B	3.0
Mm.20879	Mus musculus mRNA for leptin receptor gene-related protein	3.2
Mm.28223	inhibitor of DNA binding 4	3.4
Mm.28223	inhibitor of DNA binding 4	3.4
Mm.89495	three prime repair exonuclease 2	3.4
Mm.18213	transforming growth factor, beta 2	3.5
Mm.28119	sphingosine phosphate lyase 1	3.7
Mm.18517	RAS p21 protein activator 3	4.0
Mm.2154	Max interacting protein 1	4.1
Mm.1013	ligase I, DNA, ATP-dependent	4.3
Mm.8315	Mus musculus neural precursor cell expressed developmentally downregulated Nedd9 (Nedd9) mRNA, complete cds	5.9
Mm.22593	General transcription factor II 1	14.1
Mm.808	**mago-nashi homolog, proliferation-associated (Drosophila)**	**17.5**

Table 2. Down regulated genes list

Gene Bank ID	Gene Name	Fold Change
Mm.22675	pre B-cell leukemia transcription factor 1	0.5
Mm.4572	protein phosphatase 1, catalytic subunit, beta isoform	0.4
Mm.38248	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase)	0.4
Mm.7138	Mus musculus pleiotropic regulator 1 (PLRG1) mRNA, complete cds	0.4
Mm.5246	peptidylprolyl isomerase A	0.4
Mm.6417	CD24a antigen	0.4
Mm.2374	tachykinin 2	0.0
Mm.8156	RAD51 associated protein 1	0.3
Mm.4053	talin	0.3
Mm.27271	Mus musculus tbc1 mRNA, complete cds	0.3
Mm.16549	DNA polymerase delta 1, catalytic domain	0.3
Mm.3860	receptor-like tyrosine kinase	0.3
Mm.16347	neuropeptide nociceptin 1	0.3
Mm.980	tenascin C	0.4
Mm.10211	CD39 antigen-like 4	0.4
Mm.18213	transforming growth factor, beta 2	0.4
Mm.8858	RAR-related orphan receptor alpha	0.4
Mm.40511	erythrocyte protein band 4.1-like 3	0.4
Mm.18268	CDC-like kinase 2	0.4
Mm.3644	brain lipid binding protein	0.4
Mm.4479	RalBP1 associated Eps domain containing protein	0.4

deprivation. And ULK family genes are known genes that involved in autophagy. Autophagy is very similar process to apoptosis. Therefore we focused ULK2 genes. To characterize the function of ULK2 gene in apoptosis and autophagy, the full ULK2 gene was over-expressed in the BC3H1 cells. We applied MTT assay for measurement of cell viability. For 48 h after the transfection, cell death was observed in ULK2 overexpressed cells. As shown in Fig. 2, compared with vector transfected cells, 36% of the cells were dead by overexpressioni of ULK2. Also we performed FACS analysis to confirm apoptosis by ULK2 gene. Cells were incubated with Annexin-FITC in a buffer containing 7-Amino-

Table 3. Apoptosis related candidate gene list

Gene Bank Number	Gene Description	Fold change	Function
Mm.27664	UNC-51-like kinase (ULK) 2	2.4	serine/threonine kinase activity
Mm.19806	Histone deacetylase 2 (HDAC 2)	2.7	Regulator of transcription, DNA dependent
Mm.808	*Mago-nashi* homolog, proliferation-associated (*Drosophila*) (MAGOH)	17.5	oogenesis molecular function unknown
Mm.622	Suppressor of Ty 4 homolog (*S. cerevisiae*)	2.2	Transcription

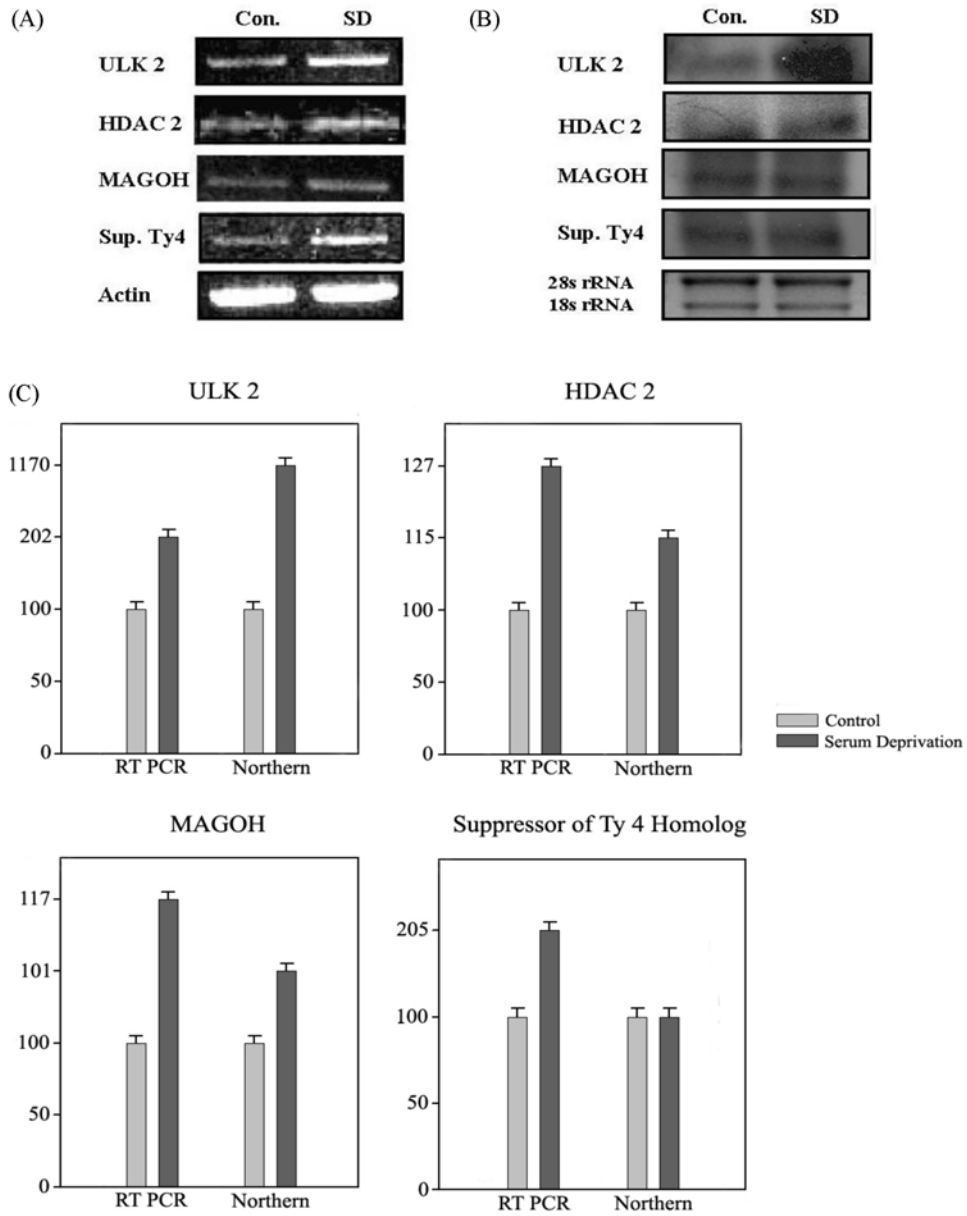

Fig. 1. Confirmation of the differential expression of the PCD related genes in RNA level. (A) RT PCR analysis of 4 selected genes. (B) Northern blot analysis of 4 selected genes. (C) Quatitation graph of RT data and Northern blot data. The RNA expression levels were quantitated by Multiguage program (Fujifilm Worldwide).

Actinomycin D (7-AAD) and analyzed in FACScan analyzer. Untreated cells were primarily Annexin-FITC and 7-AAD negative, representing that they were viable and not undergoing apoptosis. After a 48 h transfection with ULK2,

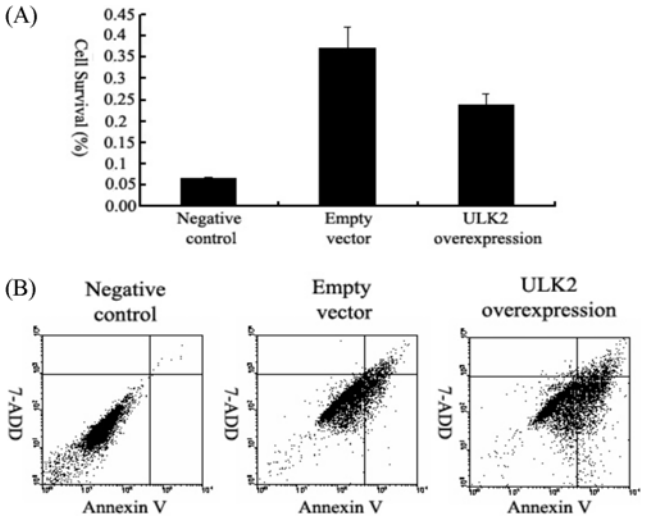

Fig. 2. Over-expressed ULK2 induced cell death. (A) Measurement of cell viability using MTT assay. (B) Annexin-FITC is a tool for identifying cells that are undergoing apoptosis. The cytogram of cells undergoing apoptosis show the early apoptotic cells in the lower right quadrant being Annexin-FITC (X axis) positive and 7-AAD (Y axis) negative; late apoptotic or necrotic cells are in the upper right quadrant being Annexin-FITC positive and 7-AAD positive; live cells in the lower left quadrant being negative for both probes.

37.26% of the cells were undergoing apoptosis and 7.54% of the cells were going through either late apoptosis or necrosis. However, in empty vector transfected cells, 79.64% of the cells lived, and only 15.22% of the cells were showing apoptosis (Fig. 2b). To identify the relation with apoptosis and ULK2 gene in molecular level, we examined the level of RNA expression of several genes related with apoptosis and autophagy. The expression of represent apoptosis related genes - BAD, PCD8, caspase 3 and caspase 8 is increased. But BAX was not increased. The expression of GAPDH that involved in both autophagy and apoptosis is increased. c-jun gene is reported to be a neuronal apoptosis-related gene (Eilers *et al*., 1998). As shown in Fig. 3, the c-jun expression is increased slightly.

Discussion

Previous study showed the morphological change due to the neuronal death in response to the serum deprivation for 24 h. Here, we demonstrated that the apoptosis-related genes are already activated within 24 h of the serum deprivation. Regarding caspase 3, the caspase 3 activity was highest after 8 h of the serum deprivation (data not shown). Because Caspase 3 is a down-stream caspase in the apoptosis pathway, this shows that the upstream molecules in the neuronal apoptosis pathway begun to be activated within 8 h of the serum deprivation. For screening of the apoptosis related genes in brain in the early period, we used the 2.3K mouse cDNA microarray.

To confirm the microarray results, we performed RT-PCR and Northern blot analysis. The overall result of the RT-PCR and northern blot analyses support the microarray data that indicate the expression of the selected genes associated in apoptotic cells deprived of serum. We were able to detect the differential expression of four genes that are involved in the serum deprivation-induced apoptosis.

Among the selected four genes, HDAC 2 is a member of the histone deacetylase family that mediates the transcriptional repression. According to present study, HDAC 2 regulates the activity of NF-κB (Ashburner *et al*., 2001) In addition, HDAC 2 regulates the growth with Sin3 by p33 (Kuzmichev *et al*., 2002). Especially, HDAC2 is related with regulation of cell cycle and apoptosis in cancer. As resent report, inhibition of HADC2 increases apoptosis through $p21^{cip1/WAF1}$ and p53 in colon cancer (Huang *et al*., 2005). In our system, HDAC2 expression was increased. These results mean that HDAC2 is involved in apoptosis regulation by tissue specific manner. Another possibility is that cells were increased HDAC2 expression for cell survival. Because we observed gene expression level after apoptosis induction for 24 h, in this condition, cells were not died perfectly. Therefore HDAC2 involved in neuronal apoptosis and neuronal survival. *Mago nashi* was initially identified as one of the posterior group genes, a set of genes that determines the localization and the fate of germ cells during the development of germ plasma in *Drosophila melanogaster*. In Drosophila, the role of *Mago-nashi* protein is the axis formation during the oogenesis, but its precise function is unclear (Newmark *et al*., 1997). In previous study, *Mago nashi* was induced in adult tissues in response to the serum stimulation. The observation that the MAGOH expression was increased in embryonic cortical cells suggests that MAGOH is associated with the cell cycle regulation in development. Another selected gene, the suppressor of Ty 4 homolog protein is identified as the homolog of yeast SPT (suppressor of Ty) gene (Chiang *et al*., 1998). The known function of the suppressor Ty 4 homolog is the transcription elongation by interacting with RNA polymerase II (Hartzog *et al*., 1998). Our data suggests the possibility that the suppressor of Ty 4 homolog is involved in apoptosis.

ULK2 is identified as the mouse homolog of the UNC51 serine/threonine kinase of C.elegans (Yan *et al*., 1999). ULK2 is involved in the neuron elongation. Recently, ULK2 has been reported to be involved in the neuron differentiation (Tomoda *et al*., 1999). Especially according to previous study, yeast Atg1 of UNC-51 like kinase homolog is required for autophagy (Matsuura *et al*., 1997). Autophagy is known the type II cell death. Also autophagical process is prominent during developmental cell death, or in hormone-deprived tissues for eliminating cells in specific area (Gozuacik *et al*., 2004). In our system, ULK2 expression was very high in apoptosis induction by serum deprivation. This fact indicated that ULK2 is involved in autophage (type II cell death). But

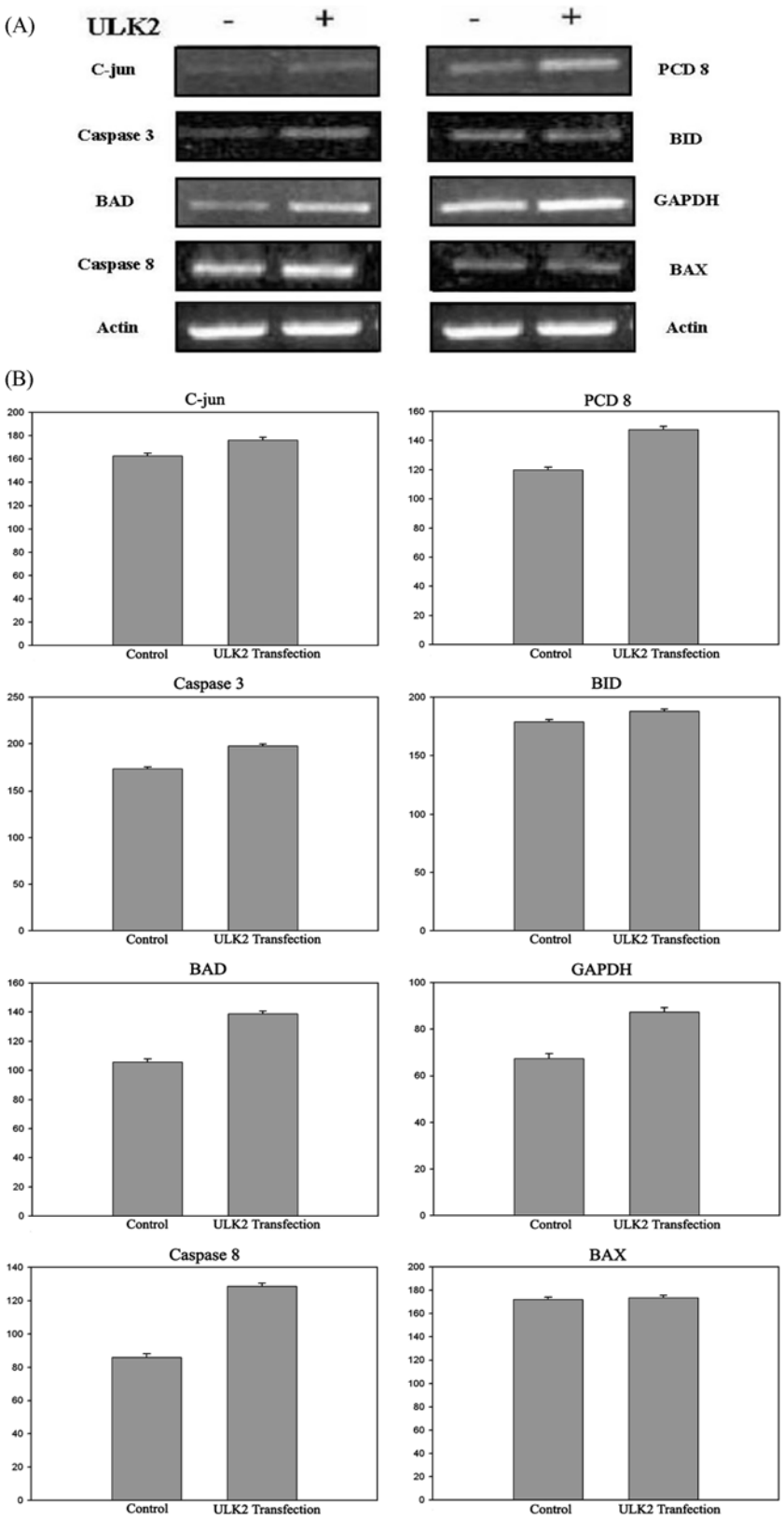

Fig. 3. The measurement of genes that were known related PCD expressed level using RT-PCR. Non-transfected cells were used as a control. (+ : RNA from transfection with ULK2, - : RNA from cells transfected with empty vector A) RT PCR analysis of known related apoptosis genes B) Quatitation graph of RT PCR analysis. The RNA expression level were quantitated by Multiguage program (Fujifilm Worldwide).

Apoptosis and autophagy are very closely interconnected types of programmed cell death. In previous study, Apoptosis and autophagy is induced at the same time in some condition like amino acid deprivation (Martinet *et al.*, 2005). To characterize the function of this gene in apoptosis and autophagy, we investigated cell death effect by ULK2 gene. We confirmed cell death by ULK2 using MTT assay and FACS analysis. Especially, FACS analysis using 7-ADD and Anexin-V is applied for confirmation of apoptosis. As shown Fig. 2. we confirmed apoptosis induction by ULK2. Therefore we examined the expression level of several genes using semi-quantitative RT-PCR. PCD8 (programmed cell death 8), BID, BAD, BAX, caspase 3 and caspase 8 is representative genes involved in apoptosis. The expression of the apoptosis inducing factor, PCD 8 and BAD were increased in response to the over-expression of ULK2. And caspase 3 and 8 also were increased by ULK2 overexpression. But BID and BAX were not changed. PCD8 and BAD are apoptosis inducing factor, these two factor overexpression is suggested that ULK 2 might be involved in apoptosis. Caspase 3 also involved in apoptosis. Therefore ULK2 might be involved in apoptosis. Caspase 8 is binding FADD and FASR. FADD induced apoptosis through caspase 8, and this gene activated both apoptosis and autophagy (Thorburn *et al.*, 2005). And in our data, GAPDH was increased. GAPDH is involved in neuronal apoptosis by regulation of p53 (Chen *et al.*, 1999). Also GAPDH is involved in autophagy pathway. In autophagy process, mTOR pathway is important role. In mTOR pathway, Atg - ULK gene is inactivated by mTOR through Akt activation. Akt also increased GAPDH and Pax gene (Shen *et al.*, 2006). Therefore in autophagical process, GAPDH is decreased by decrease of Akt. But in our data, GAPDH was increased by ULK2 overexpression. These results may be suggested that ULK2 is involved in apoptosis by p53.

In conclusion, by using the cDNA microarray technique, we have found four candidate genes that may be involved in apoptosis; UNC-51-like kinase (ULK) 2, HDAC 2, MAGOH, and the suppressor of Ty 4 protein homolog. Among these candidate genes, we focused on ULK2 gene. ULK2 is known gene that involved in autophagy - type II cell death. We investigated ULK2 function in apoptosis and autophagy. ULK2 induced apoptosis in the BC3H1 cells. And Apoptosis related genes - BAD, PCD8, caspase 3 and caspase 8 were increased by ULK2 overexpression. Especially GAPDH was increased by ULK2. Our data suggests that ULK2 may be involved in apoptosis by serum deprivation though the p53 pathway.

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