Genomics & Informatics Vol. 2(2) 67-74, June 2004

Changes of Gene Expression in NIH3T3 Cells Exposed to Osmotic and Oxidative Stresses

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Abstract

Cells consistently face stressful conditions, which cause them to modulate a variety of intracellular processes and adapt to these environmental changes via regulation of gene expression. Hyperosmotic and oxidative stresses are significant stressors that induce cellular damage, and finally cell death. In this study, oligonucleotide microarrays were employed to investigate mRNA level changes in cells exposed to hyperosmotic or oxidative conditions. In addition, since heat shock protein 70 (HSP70) is one of the most inducible stress proteins and plays pivotal role to protect cells against stressful condition, we performed microarray analysis in HSP70-overexpressing cells to identify the genes expressed in a HSP70-dependent manner. Under hyperosmotic or oxidative stress conditions, a variety of genes showed altered expression. Down-regulation of protein phosphatase1 beta (PP1 beta) and sphingosine-1-phosphate phosphatase 1 (SPPase1) was detected in both stress conditions. Microarray analysis of HSP70-overexpressing cells demonstrated that diverse mRNA species depend on the level of cellular HSP70. Genes encoding lysyl oxidase, thrombospondin 1, and procollagen displayed altered expression in all tested conditions. The results of this study will be useful to construct networks of stress response genes.

Keywords: microarray, hyperosmotic stress, oxidative stress, HSP70

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Accepted 9 May 2004

Introduction

Exposure of cells to sudden stress deleteriously affects their survival. However, a transition from a mild to harsh stress or repeated exposure to stress renders cells stress tolerance. Cells can modulate a variety of intracellular processes by changing gene expression to adapt to new stressful conditions. This conserved cellular response to harmful stimuli is referred as 'stress response'.

Hyperosmotic and oxidative stresses present significant challenges to cells. Cells respond to osmotic stress by compensational changes in cell volume, water content, and intracellular solute concentration. Some genes induced by osmotic stress have been identified (Kültz and Csonka, 1999; Cai *et al.*, 2004). High levels of reactive oxygen species (ROS) causes cellular damage and induces apoptosis, which is mediated through the mitochondria-dependent apoptotic pathway and results in the loss of mitochondrial membrane potential (Kemp *et al.*, 2003). Although the gene expressions of some genes related to stress response are known, global gene expression patterns are not.

Heat shock proteins (also known as stress proteins) have prominent roles in stress tolerance. Heat shock protein 70 (HSP70) is the most inducible protein during stress response and is highly conserved from bacteria to mammals (Tanaka *et al.*, 1988). HSP70 can be induced by various stressors including hyperthermia, hyperosmolarity, oxidative stress, heavy metals, and amino acid analogues (Morimoto *et al.*, 1992; Abe *et al.*, 1998; Fukamachi *et al.*, 1998). The induction of HSP70 has been shown to enhance cell survival after exposure to harmful stimuli (Jäättelä, 1999). HSP70 has an important function as a molecular chaperone (Hartl, 1996) and an anti-apoptotic molecule (Li *et al.*, 2000a; Beere *et al.*, 2000; Saleh *et al.*, 2000).

In this study, to profile global gene expression under hyperosmotic or oxidative stress conditions, oligonucleotide array analyses were performed. Since HSP70 plays an essential role in the acquisition of stress tolerance, altered gene expressions were also analyzed in HSP70-overexpressing cells. We found that a number of genes altered their expressions in response to hyperosmotic or oxidative stress, and the HSP70 level in a cell. These kinds of altered gene expressions act as countermeasures against stress, and help maintain homeostasis.

Materials and Methods

Cell culture and treatment

NIH3T3 and HSP70-overexpressing NIH3T3 cells were grown in DMEM media supplemented with 10 % heat-inactivated fetal bovine serum and 50 μg/ml penicillin and streptomycin in a 5 % CO_2 incubator at 37℃. For hyperosmotic or oxidative stress conditions, cells were kept in media containing 130 mM sodium chloride or 1.0 mM H_2O_2 for 2 hours. For heat shock condition, culture plates containing cells were tightly wrapped with parafilm and immersed in a water bath at the indicated temperature for 30 min, then kept in CO_2 incubator at 37℃ for indicated times.

Preparaton of RNA

Total RNA was isolated using Trizol reagent (Life Technologies, Gaithersburg, MD) according to the manufacturer's instructions. The purity was assessed by the examination of 18S and 28S bands after gel electrophoresis, and from the A_{260}/A_{280}.

Oligonucleotide array hybidization and data analysis

First strand cDNA were synthesized using 100 μg of total RNA with oligo dT primer and reverse tranacriptase in the presence of cyanine Cy_3 or Cy_5 dye at 42℃ for 1h. They were hybridized overnight at 42℃ to the MAGIC Oligo-Mouse 11K® chip (Macrogen Inc., Seoul, Korea) according to the protocol recommended by manufacturer. The slides were scanned with Array scanner generation III (Molecular dynamics, UK). The images were analyzed using Image 5.5® software (Biodiscovery, USA).

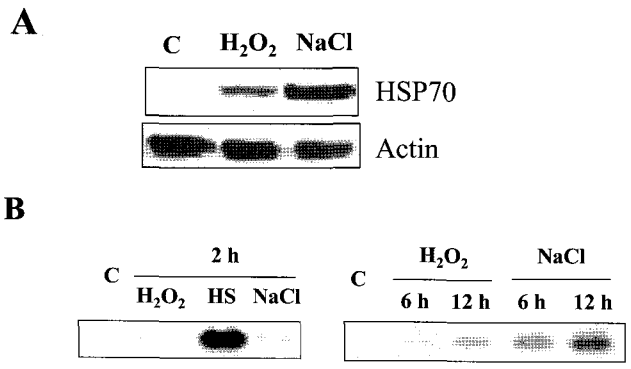

Fig. 1. Induction of HSP70 by hyperosmotic and oxidative stressors. A. Western blot analysis. NIH3T3 cells were incubated in media containing 130 mM NaCl or 1 mM H_2O_2 for 12 h. Cell lysates were separated on SDS-PAGE gel. HSP70 levels were detected using anti-HSP70 antibody. B. Northern blot analysis. Cells were exposed to hyperosmotic or oxidative stresses during the indicated time periods (2h, 6h or 12 h). Heat shock (HS) used to show immediate induction of HSP70 by heat shock in contrast to hyperosmotic or oxidative stresses. For heat treatment, NIH3T3 cells were kept in 42℃ water bath for 30 min, and then recovered in 37℃ CO_2 incubator for 2 h. Total RNAs were isolated from heat-, H_2O_2-, NaCl-treated NIH3T3 cells, and hybridized with a *hsp70* gene specific probe to detect expression level of HSP70 mRNA at each time point.

Preparation of whole cell extract and Western blot analysis

After washing with phosphate-buffered saline, the cells were scraped and collected in extraction buffer (1% Triton X-100, 1% sodium deoxycholate, 25 mM Tris-HCl, pH 7.4, 0.1% SDS, 137 mM NaCl, 1 mM EDTA). The collected cells were incubated on ice for 30 min. The lysate was centrifuged and quantitated with BCA Protein Assay Reagents (Pierce, IL, USA). The equal amounts of total proteins were separated on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane. HSP70 band was detected by sequential treatment with an anti-HSP70 antibody purchased from Santa Cruz (Santa Cruz, USA), an HRP-conjugated secondary antibody, and an enhanced chemiluminescence substrate kit.

Northern blot analysis

For Northern blot analysis, 10 μg of total RNA was fractionated on a 1% formaldehyde agarose gel, blotted onto Hybond-N+ nylon membrane (Amersham, NJ, USA). The ^{32}P-labeled probes by random priming kit (Amersham, NJ, USA) were hybridized to the blots at 62℃ in a Modified Church Hybridization Buffer (0.25 M Na_2HPO_4, 1 mM EDTA, 1% casein, 7% SDS, adjust pH to 7.4 with H_3PO_4). The blots were washed under high stringency and autoradiographed on the X-ray films (AGFA, Germany).

RT-PCR analysis

Total RNA (2 μg) was reverse transcribed from ologo-dT primers, and the resulting cDNA was amplified by PCR using gene-specific primers. Primer sequences are followed: Cdkn1a (p21) sense 5'-AGTCTCTGGTGATCT GCTGCTC, antisense 5'-GACACTGAGCAATGGCTG GACT; Tnfsf 9 sense 5'-ATGGACCAGCACACACTT, antisense 5'-GACAACCCATGGGAATGA; Nfkbia sense 5'-GCCATGTTTCAGCCAGCT, antisense 5'-GGCCAGC-GTCTGACATTA. GAPDH was internal control for amount of cDNA used for each reaction.

Results and Discussion

After NIH3T3 cells were exposed to NaCl or H_2O_2, HSP70 induction was monitored as a marker of stress response, since HSP70 is one of the most inducible proteins during stress response. HSP70 was enormously induced 10 h after treatment with either 130 mM NaCl or 1 mM H_2O_2 for 2 h (Figure 1A). Total RNAs were isolated from cells exposed to these stressful conditions. Synthesized cDNAs were hybridized to a MAGIC Oligo-Mouse 11K® chip (Macrogen Inc.). This experiment was performed in triplicate and genes with significantly and consistently altered expressions were identified. Under the hyperosmotic condition, 12 genes were up-regulated and 16 down-regulated (Table 1). The most prominently increased expression was nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha (NFKBIA). NFKBIA inhibits the action of NF-kappaB by forming a heterodimer with NF-Kappa B, which is a transcription factor for the expression of many genes involved in cell

Table 1. List of up-regulated and down-regulated genes by hyperosmolarity

GB Acc. No.	Description	Mean of fold change
Increased		
Transcription		
NM_008235	hairy and enhancer of split 1; Hes1	5.9
NM_013613	nuclear receptor subfamily 4, group a, member 2; Nr4a2	3.3
mRNA metabolism		
NM_016690	heterogeneous nuclear ribonucleoprotein D-like; Hnrpdl	2.5
rRNA processing		
BC014703	RNA, U3 small nucleolar interacting protein 2; Rnu3ip2	2.6
Signal transduction		
NM_010907	nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha; Nfkbia	18.9
NM_011333	chemokine (C-C motif) ligand 2; Ccl2	6.2
NM_011648	thyroid stimulating hormone, receptor; Tshr	2.9
Cell differentiation		
NM_008654	myeloid differentiation primary response gene 116; Myd116	3.7
Transport		
AB074017	kinesin family protein 1C; Kif1c	2.3
Miscellaneous		
NM_133662	immediate early response 3; Ier3	6.1
NM_019738	nuclear protein; Nupr1	2.4
AK012002	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39; Ddx39	2.4
Decreased		
Transcription		
NM_017373	nuclear factor, interleukin 3, regulated; Nfil3	2.5
Protein biosynthesis		
X03688	eukaryotic translation elongation factor 1 alpha 1; Eef1a1	2.4
Apoptosis		
NM_012032	tumor differentially expressed 1; Tde1	2.7
Dephosphorylation		
NM_030750	sphingosine-1-phosphate phosphatase 1; Sgpp1	4.1
M27073	protein phosphatase 1, catalytic subunit, beta isoform; Ppp1cb	2.7
Cell division/growth		
N96163	polo-like kinase 2; Plk2	2.9
Cell adhesion		
AK019448	procollagen, type III, alpha 1; Col3a1	5.0
NM_011580	thrombospondin 1; Thbs1	4.9
NM_007743	procollagen, type I, alpha 2; Col1a2	2.6
Proteolysis		
AF330052	serine caroboxypeptidase 1; Scpep1	2.7
Transport		
NM_013496	cellular retinoic acid binding protein 1; Crabp1	2.3
Ion binding		
NM_010728	lysyl oxidase; Lox	5.0
Miscellaneous		
J01420	protein 41	5.3
AJ005350	zinc finger protein 125; Zfp125	3.3
NM_024427	tropomyosin 1, alpha; Tpm1	2.4
NM_078478	growth hormone inducible transmembrane protein; Ghitm	2.3

Table 2. List of up-regulated and down-regulated genes by oxidative stress

GB Acc. No.	Description	Mean of fold change
Increased		
Transcription		
NM_009087	RNA polymerase 1-3; Rpo1-3	2.4
Cell division/growth		
NM_007669	cyclin-dependent kinase inhibitor 1a (p21); Cdkn1a	3.8
NM_008380	inhibin beta-a; Inhba	2.7
NM_018868	nucleolar protein 5; Nol5	2.1
Metabolism		
NM_016690	heterogeneous nuclear ribonucleoprotein D-like; Hnrpdl	2.4
NM_008490	lecithin cholesterol acyltransferase; Lcat	2.2
Signal transduction		
NM_013602	metallothionein 1; Mt1	5.6
NM_011333	chemokine (C-C motif) ligand 2; Ccl2	2.7
Dephosphorylation		
NM_013935	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a; Ptpla	2.5
Cell adhesion		
BC019746	adhesion regulating molecule 1; Adrm1	2.6
Miscellaneous		
NM_013562	interferon-related developmental regulator 1; Ifrd1	2.6
NM_021885	tubby candidate gene; Tub	2.2
Decreased		
Transcription		
AF062567	trans-acting transcription factor 3; Sp3	3.2
NM_008241	forkhead box g1; Foxg1	2.2
Biosynthesis		
D26047	phosphatidylinositol glycan, class A; Piga	2.4
U89416	eukaryotic translation elongation factor 2; Eef2	2.4
Cell division/growth		
NM_010128	epithelial membrane protein 1; Emp1	2.5
NM_009465	axl receptor tyrosine kinase; Axl	2.3
NM_007900	ect2 oncogene; Ect2	2.2
Oxidative stress		
NM_007770	cone-rod homeobox containing gene; Crx	7.8
NM_010431	hypoxia inducible factor 1, alpha subunit; Hif1a	3.6
Signal transduction		
NM_020510	frizzled homolog 2; Fzd2	3.5
NM_009706	Rho GTPase activating protein 5; Arhgap5	2.8
Dephosphorylation		
NM_030750	sphingosine-1-phosphate phosphatase 1; Sgpp1	3.6
M27073	protein phosphatase 1, catalytic subunit, beta isoform; Ppp1cb	3.0
Cell adhesion		
NM_011580	thrombospondin 1; Thbs1	5.0
M14423	procollagen, type I, alpha 1; Col1a1	3.6
AB041350	procollagen, type IV, alpha 5; Col4a5	3.4
NM_007743	procollagen, type I, alpha 2; Col1a2	2.9
Protein folding		
NM_020031	prefoldin 5; Pfdn5	2.1
Endocytosis		
NM_007616	caveolin, caveolae protein; Cav	3.6
Proteolysis		
NM_011782	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (aggrecanase-2); Adamts5	4.1
AF330052	serine caroboxypeptidase 1; Scpep1	2.3
NM_008610	matrix metalloproteinase 2; Mmp2	2.3
Nucleic acid binding		
NM_019711	RNA binding motif, single stranded interacting protein 2; Rbms2	2.8
AK012998	RIKEN cDNA 2810409H07	2.1
Ion binding		
NM_010728	lysyl oxidase; Lox	5.2
Miscellaneous		
AY074887	cleft palate-related protein 1; Mcpr1	9.5
AB041540	transmembrane 4 superfamily member 10; Tm4sf10	4.6
AK019938	RIKEN cDNA 5330439B14 gene	4.1
AK002818	signal sequence receptor, gamma; Ssr3	3.7
BC025904	angiopoietin-like protein 6; Angpt6	3.2
NM_015776	microfibrillar associated protein 5; Mfap5	3.1
NM_008987	pentaxin related gene; Ptx3	3.0
NM_009441	tetratricopeptide repeat domain; Ttc3	3.0
BC019435	caldesmon 1; Cald1	2.7
NM_010771	matrin 3; Matr3	2.5
AB075019	RIKEN cDNA 2610001E17gene	2.3

proliferation, differentiation, apoptosis, or metastasis (Curran *et al.,* 2002). It has been reported that the inhibition of NFKBIA phosphorylation sensitizes cells to radiation (Ding *et al.,* 2003). From our results, the most affected transcription factor might be NF-kappaB, since its inhibitor level was increased 19 fold under hyperosmotic stress. On the other hand, eukaryotic translation elongation factor 1 alpha 1 was down-regulated. It is generally known that the cells under stress suppress the de novo synthesis of protein except some stress-related proteins. Our results show that translation elongation factor 1 might be important for the suppression of protein synthesis in conditions of hyperosmotic stress. Polo-like kinase 2, which regulates cell cycle was also down-regulated (Ma *et al.,* 2003). Protein phosphatase 1 beta isoform (PP1 beta) and sphingosine-1-phosphate phosphatase 1 (SPPase1) were decreased by 2.7 and 4.1 fold, respectively. The fine regulation of protein phosphorylation is essential to transmit proper signals intracellularly. The expression level of PP1 can be specifically affected by various cellular stresses (Amador *et al.,* 2004). These authors suggested that the monitoring of PP1 expression could be a useful diagnostic tool for cellular stress, since alterations of PP1 expression is an immediate early stress response, which occurs before heat shock protein alterations. From our chip data, it was supported that PP1 could be a sensitive stress marker molecule like HSP70. When we observed the induction levels of HSP70 mRNA 2h after heat shock, heat shock markedly induced HSP70 mRNA as shown in Figure 1B (left panel) Whereas HSP70 mRNA was not detected within 2 h under oxidative stress condition, and was induced extremely low level of HSP70 mRNA 2 h under hyperosmotic stress (Figure 1B, left panel). In both cases, HSP70 mRNA was detectable 6 h or 12 h after treating with stressors (Fig 1B, right panel). Our result suggests that immediate induction of HSP70 seems to depend on the type of stress. Sphingosin-1-phosphate (SPP) has multiple functions as a second messenger in cell proliferation, angiogenesis, and apoptosis (Johnson *et al.,* 2003). The loss of SPPase results in cellular tolerance to stress, and the overexpression of SPPase provokes apoptosis (Mandala, 2001). Therefore, the regulation of the sphingosin-1-phosphate level by reducing SPPase1 expression could be an important response to hyperosmotic stress.

In oxidatively stressed cells, 12 genes were up-regulated and 36 down-regulated (Table2). Cyclin dependent kinase inhibitor 1a (CDKN1A, p21) was increased 3.8 fold. Cell cycle arrest is correlated with down-regulation of cyclin D1 and up-regulation of CDKN1A (Todd *et al.,* 2004). Acutely challenged cells usually show cell cycle arrest, and adopt a stress response to overcome the applied stress. Rapidly increased CDKN1A might have a major role on cell cycle arrest in oxidative condition. We observed obvious increases in metallothionein 1 (MT1) expression. MT1 is small sulfhydryl-rich protein involved in cellular protection, probably by inhibiting oxidative stress (Wanpen *et al.,* 2004; Li *et al.,* 2004). Decreases in PP1 beta (3.0 fold) and SPPase1 (3.6 fold) were also observed under oxidative stress condition. Genes encoding lysyl oxidase (LOX), thrombospondin 1 (TSP1), and several types of procollagen were down-

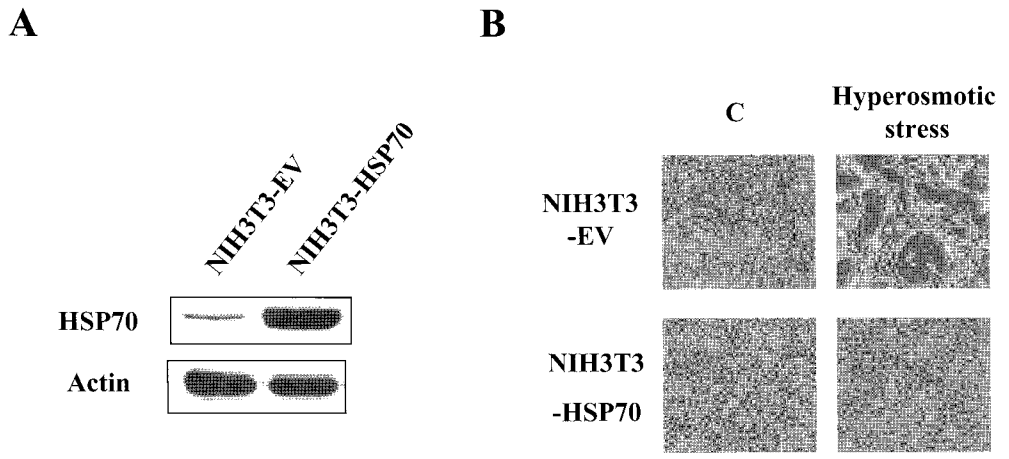

Fig. 2. Protective effect of HSP70 against hyperosmolarity-induced cell death. A. Expression levels of HSP70 in empty vector-transfected (NIH3T3-EV) and HSP70-transfected (NIH3T3-HSP70) cells. NIH3T3 cells were stably transfected with empty vector or pCMV-HSP70 recombinant vector. HSP70 levels were detected by western blotting. B. Protective role of HSP70 under lethal stress condition. Cells were exposed to a lethal concentration of NaCl (300 mM) and cell viability was observed.

Table 3. List of up-regulated and down-regulated genes in HSP70-overexpressing cells

GB Acc. No.	Description	Mean of fold change
Increased		
DNA replication		
NM_019716	origin recognition complex, subunit 6-like; Orc6l	2.6
NM_023595	deoxyuridine triphosphatase; Dutp	2.2
Transcription		
NM_008818	placentae and embryos oncofetal gene; Pem	8.0
NM_009087	RNA polymerase 1-3; Rpo1-3	2.4
AF222443	NK1 transcription factor related, locus 2; Nkx1-2	2.1
Chromatin remodeling		
NM_019673	expressed sequence C79802	2.1
RNA processing		
NM_016784	pleiotropic regulator 1, PRL1 homolog; Plrg1	2.3
Oxidative stress		
NM_008160	glutathione peroxidase 1; Gpx1	2.2
Apoptosis		
NM_009404	tumor necrosis factor (ligand) superfamily, member 9; Tnfsf9	3.9
Development		
NM_010760	mago-nashi homolog, proliferation-associated; Magoh	2.3
NM_031260	moloney leukemia virus 10-like 1; Mov10l1	2.1
Poteolysis		
AK011974	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14; Psmd14	3.5
NM_011593	tissue inhibitor of metalloproteinase; Timp	2.4
Transport		
NM_011402	solute carrier family 34 (sodium phosphate), member 2; Slc34a2	2.5
NM_007507	ATP synthase, H+ transporting, mitochondrial F1F0 complex, subunit e; Atp5k	2.4
Miscellaneous		
AK009946	RIKEN cDNA 2310056P07 gene	3.6
NM_031161	cholecystokinin; Cck	3.0
NM_019814	hypoxia induced gene 1; MGI:1930666	3.0
BC021588	RIKEN cDNA 2310003F16 gene	2.3
Decreased		
Transcription		
NM_009343	PHD finger protein 1; Phf1	3.5
NM_010828	Cbp/p300-interacting transactivator, with Glu/Asp-rich 3.1 carboxy-terminal domain, 2; Cited2	3.1
D85612	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3; Nfat3	2.7
Protein biosynthesis		
U89416	eukaryotic translation elongation factor 2; Eef2	6.0
M74012	seryl-aminoacyl-tRNA synthetase 1; Sars1	3.1
Cell division/growth		
NM_008512	low density lipoprotein receptor-related protein 1; Lrp1	4.2
NM_008344	insulin-like growth factor binding protein 6; Igfbp6	2.9
NM_007631	cyclin D1; Ccnd1	2.8
Signal transduction		
AF075456	endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor 2; Edg2	2.7
L29278	signal transducer and activator of transcription 3; Stat3	2.9
Cell adhesion		
NM_011580	thrombospondin 1; Thbs1	7.7
M14423	procollagen, type I, alpha 1; Col1a1	3.0
Proteolysis		
NM_011782	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (aggrecanase-2); Adamts5	4.6
NM_023476	lipocalin 7; Lcn7	2.5
Transport		
NM_013496	cellular retinoic acid binding protein i; Crabp1	5.1
AF353671	filamin alpha; Flna	3.4
NM_011404	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5; Slc7a5	3.0
AK014235	exportin, tRNA(nuclear export receptor for tRNAs); Xpot	2.3
Ion binding		
NM_007594	calumenin; Calu	4.5
NM_010728	lysyl oxidase; Lox	3.0
Miscellaneous		
AY074887	cleft palate-related protein 1; Mcpr1	4.4
L27153	kinesin family member 5B; Kif5b	3.8
NM_009382	thymus cell antigen 1, theta; Thy1	3.7
NM_009468	dihydropyrimidinase-like 3; Dpysl3	2.8

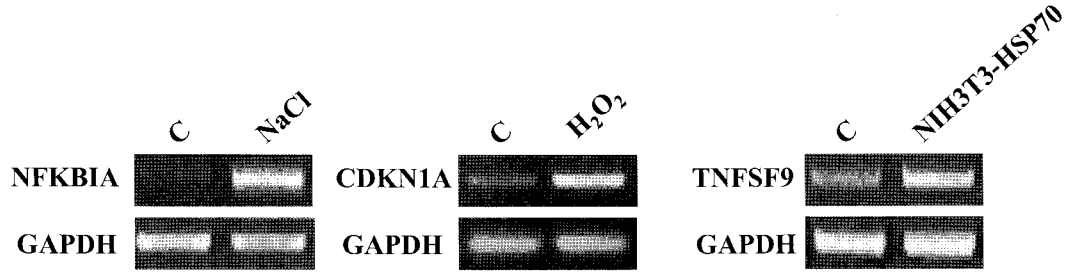

Fig. 3. RT-PCR of the genes regulated by hyperosmolarity, oxidative stress, or HSP70- overexpression. RNA was isolated from NIH3T3 cells treated with 130 mM NaCl (A), 1 mM H_2O_2 (B), or stably transfected with *hsp70* gene. NFKBIA stands for nuclear factor of kappa light chain gene enhancer in B-cells inhibitor alpha. CDKN1A and TNFSF9 mean cyclin-dependent kinase inhibitor 1a (p21) and tumor necrosis factor superfamily member 9, respectively.

regulated under hyperosmotic and oxidative stress. LOX is an enzyme that initiates the covalent crosslinking of polypeptide chains within extracellular matrix (ECM) proteins, collagen and elastin. LOX expression was regulated by diverse stimuli including growth factors, angiotensin II, retinoic acid, and shear stress (Kagna and Li, 2003). LOX has roles in stress fiber formation and focal adhesion assembly (Li *et al.*, 2000b). Moreover, LOX is one of the genes known to be dysregulated in diseases associated with oxidative stress (Li *et al.*, 2004). It has been suggested that LOX may have other important biological functions in addition to its role as an ECM cross linker. Another ECM molecule, TSP1 expression was decreased about 5 fold by both hyperosmotic and oxidative stress. TSP1 can exert cellular adhesive changes and pro- or anti-angiogenic effects. It was reported that TSP1 might be involved in cellular adaptation to cellular damage (Ann Elzie and Murphy-Ullrich, 2004; Espey *et al.*, 2003). Collagens are major constituents of the ECR, and form large superfamilies. They are vital components of connective tissue, and are centrally involved in the formation of fibrillar and microfibrillar networks of the ECM, and in basement membranes as well as of other structures of the ECM (Gelse *et al.*, 2003). Changes in the balance of ECR molecules can lead to collagen-related diseases (Siwik and Colucci, 2004). In this study, we found that cells under hyperosmotic or oxidative stress rapidly and obviously change the expression levels of ECM proteins.

Cells under stress are able to induce stress response to prevent cellular damage by modulating gene expression. HSP70 is one of the most inducible genes involved in stress response. We generated HSP70-overexpressing NIH3T3 cells (NIH3T3-HSP70), and observed cell viability comparing to empty vector transfected cells (NIH3T3-EV) under lethal hyperosmotic condition (Figure 2). Since HSP70 has pivotal roles in stress tolerance, we wanted to know which gene expressions are affected by changes in the intracellular level of HSP70. Microarray analysis showed that 19 genes were up-regulated and 24 down-regulated in HSP70-overexpressing cells (Table 3). The overexpression of HSP70 influenced the expressions of various molecules involving transcription, translation, cell division and growth, transport, proteolysis, cell adhesion and others. The signal transducing molecule, STAT, was down-regulated 2.9 fold. It suggests that HSP70 directly or indirectly affects STAT expression and mediates their signal transducing pathways. Interestingly, LOX (3.1 folds), TSP1 (7.7 folds), and procollagen type I (3.0 folds) were commonly down-regulated in HSP70-overexpressing NIH3T3 cells. The ECM is important for many cellular processes such as cell division, migration, differentiation, and death. Abnormalities of the ECM might be closely linked to diseases such as rheumatoid arthritis, restenosis, tumor invasion and metastasis, and atherosclerosis (Lee *et al.*, 2004). This means that the primary molecules targeted by hyperosmotic and oxidative insults are constituents of the ECM, and that HSP70 has key roles in ECM integrity. HSP70 is being considered for possibly use as a therapeutical molecule in diseases related with these protein families. We performed RT-PCR to confirm our microarray results as shown in Figure 3. These results showed that NFKBIA in hyperosmotic conditions, CDKN1A in oxidative conditions, and TNFSF9 in HSP70-overexpressing cells were up-regulated, in accord with the microarray results.

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