Identification of common gene networks responsive to mild hyperthermia in human cancer cells

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Abstract. Hyperthermia (HT) has been used as a possible treatment modality for various types of malignant tumors. Due to its pleiotropic effects, its combined use with radiotherapy and/or chemotherapy has proven to be beneficial. However, the molecular mechanisms underling the cellular responses to heat stress remain unclear. Therefore, the aim of this study was to identify common gene expression patterns responsive to mild HT (MHT) in human cancer cells. HeLa human cervical squamous cell carcinoma (SCC) and HSC-3 human oral SCC cells were exposed to MHT at 41˚C for 30 min, followed by culture at 37˚C for 0-24 h. MHT did not affect cell viability or the cell cycle. GeneChip microarray analysis clearly revealed that many probe sets were differentially expressed by a factor of ≥1.5 in both cell lines following exposure to MHT. Of the many differentially expressed probe sets, 114 genes were found to be commonly upregulated in both HeLa and HSC-3 cells, and two significant gene networks were obtained from the commonly upregulated genes. Gene network A included several heat shock proteins, as well as BCL2-associated athanogene 3 (BAG3), and was found to be mainly associated with the biological functions of cellular function and maintenance. Gene network B included several anti-cell death genes, such as early growth response 1 (EGR1) and endothelin 1 (EDN1) and was found to be associated with the biological functions of cell death and survival. Real-time quantitative polymerase chain reaction demonstrated that the gene expression patterns of the 12 genes selected were consistent with the microarray data in four cancer cell lines. These findings may provide further insight into the detailed molecular mechanisms of the MHT response in cancer cells.

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Abbreviations: BAG3, BCL2-associated athanogene 3; BCL2, B-cell CLL/lymphoma 2; BCL6, B-cell CLL/lymphoma 6; BHLHE40, basic helix-loop-helix family, member e40; BMP2, bone morphogenetic protein 2; CTGF, connective tissue growth factor; DMEM, Dulbecco's modified Eagle's medium; DNAJB1, DnaJ (Hsp40) homolog, subfamily B, member 1; DNAJB4 DnaJ (Hsp40) homolog, subfamily B, member 4; DUSP1, dual specificity phosphatase 1; EDN1, endothelin 1; ERG1, early growth response 1; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HSF1, heat shock transcription factor 1; HSP, heat shock protein; HSPA1A/B, heat shock 70 kDa protein 1A/B; HSPA1H, heat shock 105/110 kDa protein; HSPA1L, heat shock 70 kDa protein 1-like; HSPA4L, heat shock protein 4 like; HSPA6, heat shock 70 kDa protein 6 (HSP70B'); HSPB1, heat shock 27 kDa protein 1; HT, hyperthermia; MHT, mild hyperthermia; MXD1, MAX dimerization protein 1; PI, propidium iodide; qPCR, quantitative polymerase chain reaction; SCC, squamous cell carcinoma; SOCS3, suppressor of cytokine signaling 3; SOX6, SRY (sex determining region Y)-box 6

Key words: mild hyperthermia, microarray, gene network, cancer cells

Introduction

Hyperthermia (HT) is a well-established treatment modality that is used alone or in combination with other therapies, such as radiotherapy and chemotherapy, for the treatment of a variety of tumors (1). It has been indicated that only cancer cells treated at temperatures >42.5˚C, the inflection point of HT, will undergo cell death as the temperature increases, whereas temperatures in the mild hyperthermia (MHT) range <42.5˚C induce little or no cytotoxicity (2). However, HT monotherapy, maintaining a cancer temperature >42.5˚C for direct cytotoxicity, has been associated with higher toxicity and less efficacy against cancer. Therefore, MHT, in combination with radiotherapy and/or chemotherapy, has been used for the treatment of various types of cancer, and its antitumor effects have been verified by several clinical trials (3-7). In addition, recent studies have demonstrated that an antitumor immune system including heat shock protein (HSP) chaperone tumor antigens may be effective as a cancer immunotherapy based on MHT (8,9).

The heat shock response, a universal cellular response to elevated temperatures, is a very important cellular event for cell adaptation. It has been well recognized that heat elicits a wide variety of stress responses, such as the induction of HSPs, DNA and RNA damage, protein aggregation, cell growth arrest and cell death (10-12). HSPs, which are are induced by heat in particular, behave as strong cytoprotective molecules preventing cell death (12) and participate in thermoresistance (13,14).

Although heat affects several biological processes, the overall responses to heat in mammalian cells are not yet completely understood. To this end, a number of studies have found that the most effective approach for investigating heat responses is to use global-scale microarrays and bioinformatics tools. Several genes and biological functions in a wide variety of cell types affected by heat stress have thus been reported (15-27).

In the present study, in order to further elucidate the molecular mechanisms underlying cellular responses to MHT in cancer cells, we compared the gene expression profiles by using high-density oligonucleotide microarrays and computational gene expression analysis tools in two human cancer cell lines, HeLa cervical squamous cell carcinoma (SCC) and HSC-3 oral SCC cells, exposed to MHT at 41˚C. Moreover, common expression patterns responsive to MHT in two other human cell lines, MCF-7 breast adenocarcinoma and HO-1-N-1 oral SCC, in addition to the HeLa and HSC-3 cell lines were determined by real-time quantitative polymerase chain reaction (qPCR).

Materials and methods

Cell culture. The human oral SCC cell lines, HSC-3 (derived from tongue carcinoma) and HO-1-N-1 (derived from buccal mucosa) were obtained from the Human Science Research Resources Bank of the Japan Health Sciences Foundation (Tokyo, Japan). The human cervical SCC cell line, HeLa, and the breast adenocarcinoma cell line, MCF-7, were provided by the RIKEN BioResource Center through the National BioResource Project of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) (Tsukuba, Japan). The HeLa and MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Life Technologies Co., Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS). The HSC-3 and HO-1-N-1 cells were cultured in Eagle's minimum Essential medium (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and DMEM/Ham's F-12 medium (Wako Pure Chemical Industries, Ltd.), respectively, each supplemented with 10% FBS. The cells were maintained at 37˚C in a humidified atmosphere with 5% CO_2 and 95% air.

Exposure to MHT. The cells were exposed to MHT by immersing plastic culture vessels containing the attached cells in a water bath at 41˚C for 30 min. Following exposure to heat, the cells were incubated for 0-24 h at 37˚C.

Analyses of cell viability and cell cycle. For cell viability, the trypan blue dye exclusion test was performed. The cells excluding the dye were counted using a hematocytometer. For the cell cycle, cells were fixed with 70% ice-cold ethanol and then treated with RNase A and propidium iodide (PI). The samples were run on an Epics XL flow cytometer (Beckman Coulter, Fullerton, CA, USA) as previously described (28).

RNA isolation. Total RNA was extracted from the cells using an RNeasy Total RNA Extraction kit (Qiagen, Valencia, CA, USA) along with On-Column DNase I treatment. The RNA concentration was measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). RNA integrity was determined using a Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CA, USA). RNA samples with RNA integrity number (RIN) values >9.5 were considered acceptable.

Global-scale gene expression analysis. Global-scale gene expression analysis was performed using a GeneChip® microarray system with a Human Genome U133-plus 2.0 array, which was spotted with 54,675 probe sets (Affymetrix Inc., Santa Clara, CA, USA) according to the manufacturer's instructions. In brief, 500 ng of total RNA were used to synthesize cRNA with a GeneChip® 3' IVT Express kit (Affymetrix Inc.). Following fragmentation, biotin-labeled cRNA was hybridized to the array at 45˚C for 16 h. The arrays were washed, stained with streptavidin-phycoerythrin, and scanned using a probe array scanner. The obtained hybridization intensity data were analyzed using GeneSpring® GX (Agilent Technologies Inc.) to extract the significant genes. To examine gene ontology, including biological processes, cellular components, molecular functions and gene networks, the obtained data were analyzed using Ingenuity® Pathway Analysis tools (Ingenuity Systems Inc., Mountain View, CA, USA), a web-delivered application that enables the identification, visualization and investigation of molecular interaction networks in gene expression data (29,30).

Real-time qPCR assay. The reverse transcriptase reaction was carried out with total RNA using a random 6-mer and an oligo(dT) primer. Real-time qPCR was performed on an Mx3005P real-time PCR system (Agilent Technologies Inc.) using SYBR PreMix ExTaq (Takara Bio Inc., Shiga, Japan) or Premix Ex Taq (for the use of TaqMan probes; Takara Bio Inc.). The specific primer and probe sequences for B-cell CLL/lymphoma 2 (BCL2)-associated athanogene 3 (BAG3), B-cell CLL/lymphoma 6 (BCL6), bone morphogenetic protein 2 (BMP2), connective tissue growth factor (CTGF), DnaJ (Hsp40) homolog, subfamily B, member 1 (DNAJB1), endothelin 1 (EDN1), early growth response 1 (ERG1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), heat shock 70 kDa protein 1A/B (HSPA1A/B), heat shock 105/110 kDa protein (HSPA1H), heat shock 70 kDa protein 6 (HSP70B') (HSPA6), MAX dimerization protein 1 (MXD1) and suppressor of cytokine signaling 3 (SOCS3) are listed in Table I. GAPDH was used as a control for normalization.

Statistical analysis. Data are presented as the means ± SD. Differences between pairs of data sets were analyzed using the Student's t-test, with values of $P<0.05$ considered to indicate statistically significant differences.

Results

Effects of MHT on cell viability and the cell cycle in cancer cell lines. We used a trypan blue dye exclusion test to assess cell viability. Fig. 1A and B indicate the number of HeLa and HSC-3 cells following exposure to MHT at 41˚C for 30 min followed by culture at 37˚C for 24 h. MHT did not inhibit cell viability compared to the control (37˚C treatment) in either cell line. Moreover, the cell cycle distribution was monitored by PI staining with flow cytometry. The percentages of control HeLa and HSC-3 cells in the G0/G1, S and G2/M phase were 26.9±4.8, 18.5±3.3 and 52.3±1.2% for the HeLa cells and 17.5±4.2, 19.3±2.5 and 62.2±3.4% for the HSC-3 cells

Table I. Nucleotide sequences of primers and TaqMan probes for target genes.

Gene	Orientation	Nucleotide sequence (5'-3')	GenBank accession no.
BAG3	Sense	CGACCAGGCTACATTCCCAT	NM_004281
	Antisense	TCTGGCTGAGTGGTTTCTGG	
BCL6	Sense	AATCGTCTCCGGAGTCGAGACA	NM_002357
	Antisense	TGATGTTGCCCTCCCGCAAA	
BMP2	Sense	ATGCTGTGTCCCGACAGAAC	NM_001200
	Antisense	AACCCTCCACAACCATGTCC	
CTGF	Sense	CTGCAGGCTAGAGAAGCAGA	NM_001901
	Antisense	CCGTCGGTACATACTCCACA	
DNAJB1	Sense	ACCCGGACAAGAACAAGGAG	NM_006145
	Antisense	GCCACCGAAGAACTCAGCAA	
EDN1	Sense	CTTGCCAAGGAGCTCCAGAA	NM_001955
	Antisense	GGCTTCCAAGTCCATACGGAA	
HSPA1A/B	Sense	AGGTGCAGGTGAGCTACAAG	NM_005346
	Antisense	ATGATCCGCAGCACGTTGAG	
HSPH1	Sense	ACCATGCTGCTCCTTTCTCC	NM_006644
	Antisense	CTGGGTTTTCTGGTGGTCTC	
HSPA6	Sense	GGCCATGACCAAGGACAACA	NM_002155
	Antisense	AACCATCCTCTCCACCTCCT	
MXD1	Sense	AGACGGGCTCATCTTCGCTT	NM_002357
	Antisense	GCTGAAGCTGGTCGATTTGGTG	
SOCS3	Sense	TCTGTCGGAAGACCGTCAAC	NM_003955
	Antisense	TAAAGCGGGGCATCGTACTG	
EGR1	Sense	GGAGCAAAATAAGGAAGAG	NM_001964
	Antisense	GAGAGTTCAGATTTTGTTTTAG	
	Probe	FAM-AGCTGAGCTTCGGTTCTCCA-TAMRA	
GAPDH	Sense	AAGGACTCATGACCACAGTCCAT	NM_002046
	Antisense	CCATCACGCCACAGTTTCC	
	Probe	FAM-CCATCACTGCCACCCAGAAGACTGTG-TAMRA	

BAG3, BCL2-associated athanogene 3; BCL6, B-cell CLL/lymphoma 6; BMP2, bone morphogenetic protein 2; CTGF, connective tissue growth factor; DNAJB1, DnaJ (Hsp40) homolog, subfamily B, member 1; EDN1, endothelin 1; HSPA1A/B, heat shock 70 kDa protein 1A/B; HSPA6, heat shock 70 kDa protein 6 (HSP70B'); MXD1, MAX dimerization protein ; SOCS3, suppressor of cytokine signaling 3; EGR1, early growth response 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

(means ± SD), respectively. Changes in cell cycle distribution were not observed in the cells exposed to MHT (Fig. 1C and D). Exposure to MHT at 41˚C for 30 min did not affect cell viability or the cell cycle distribution in the other cancer cell lines, MCF-7 and HO-1-N-1 (data not shown). These data demonstrated that the cells exposed to MHT at 41˚C for 30 min did not undergo cell damage and cell cycle arrest.

Global-scale gene expression analysis. Global-scale gene expression analysis was performed using a GeneChip microarray system and GeneSpring software. Of the 54,675 probe sets analyzed, many probe sets that were differentially expressed by ≥1.5-fold were detected. We identified 1,053 and 1,278 upregulated, and 701 and 673 downregulated probe sets in the HeLa and HSC-3 cells exposed to MHT at 41˚C for 30 min, respectively. Fig. 2 demonstrates the time course of changes in gene expression. In all, 248 and 52 probe sets were also found to be commonly upregulated and downregulated in these respective cell lines (Fig. 2). The complete lists of genes from the HeLa and HSC-3 cell samples have been deposited in the Gene Expression Omnibus (GEO), a public database (accession numbers: GSE43701 and GSE43862).

Identification of biological functions and gene networks. To identify the biological functions and gene networks in the differentially expressed probe sets in the HeLa and HSC-3 cells exposed to MHT, either functional category or gene network analysis was conducted by use of the Ingenuity Pathways Knowledge Base. We identified several functionally annotated genes: 494 and 637 upregulated genes, and 303 and 330 down-

Table II. Top three biological functions.

Name	P-value	Numbers of molecules
HeLa upregulated genes (494)[a]		
Cell death and survival	3.34E-05-3.39E-02	224
Gene expression	4.92E-05-3.39E-02	169
Cellular development	2.89E-04-3.39E-02	202
HeLa downregulated genes (303)[a]		
Gene expression	6.21E-06-3.57E-02	140
Cellular assembly and organization	1.00E-04-3.57E-02	90
Cellular function and maintenance	1.00E-04-3.57E-02	80
HSC-3 upregulated genes (637)[a]		
Cell death and survival	2.92E-13-2.85E-03	292
Gene expression	3.32E-13-2.71E-03	248
Cellular growth and proliferation	4.54E-10-2.99E-03	299
HSC-3 downregulated genes (330)[a]		
Cell cycle	3.44E-06-3.24E-02	102
Cell death and survival	3.31E-05-3.25E-02	148
Cellular development	9.25E-05-3.26E-02	111
Commonly upregulated genes (114)[a]		
Cellular function and maintenance	2.21E-07-1.89E-02	48
Cell death and survival	2.46E-06-2.01E-02	60
Cellular movement	2.97E-05-2.02E-02	40
Commonly downregulated genes (35)[a]		
Cell cycle	9.35E-05-4.86E-02	48
Cell death and survival	6.09E-04-4.56E-02	60
Gene expression	6.17E-04-4.86E-02	40

[a]Numbers of functionally annotated genes.

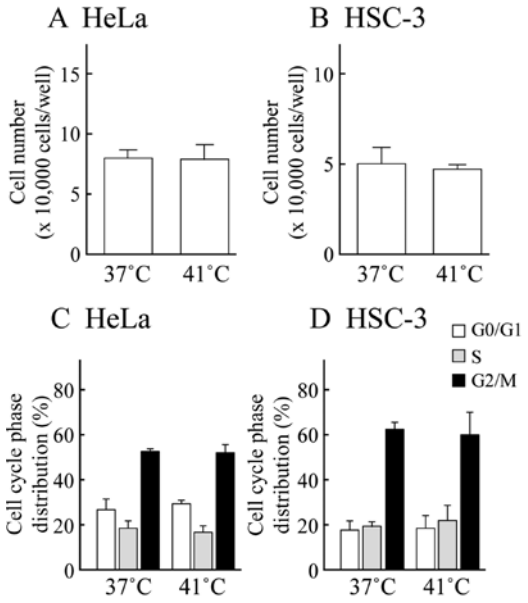

Figure 1. Effects of mild hyperthermia (MHT) on cell viability and the cell cycle in HeLa and HSC-3 cells. The cells were incubated at 37˚C (control) or 41˚C (MTH) for 30 min and then cultured at 37˚C for 24 h. (A and B) The cell viability and (C and D) cell cycle were analyzed. (A and C) HeLa cells. (B and D) HSC-3 cells. Data are presented as the means ± SD (n=4).

regulated genes in the HeLa and HSC-3 cells exposed to MHT, respectively (Table II). The top three biological functions in each group are summarized in Table II. Biological functions including cell death and survival, gene expression and cellular function and maintenance, were observed in the upregulated genes in both cell lines, and the most prominent functions of commonly upregulated genes were cellular functions and maintenance (48 genes), as well as cell death and survival (60 genes). In the downregulated genes in both cell lines, biological functions including cell cycle, gene expression and cell death and survival were observed. The specific biological functions were cell cycle (13 genes) and cell death and survival (18 genes) in commonly downregulated genes (Table II). Furthermore, we identified two significant gene networks, A and B, that were obtained from commonly upregulated genes in both cell lines (Figs. 3 and 4). Gene network A included several HSPs, such as HSPA1A/B, heat shock 70 kDa protein 1-like (HSPA1L), heat shock protein 4 like (HSPA4L), HSPA6, heat shock 27 kDa protein 1 (HSPB1), HSPH1, DNAJB1, and DnaJ (Hsp40) homolog, subfamily B, member 4 (DNAJB4), as well as BAG3, and was associated with the biological functions of cellular function and maintenance (Fig. 3). Gene network B included ten genes: BCL6, basic helix-loop-helix family, member e40

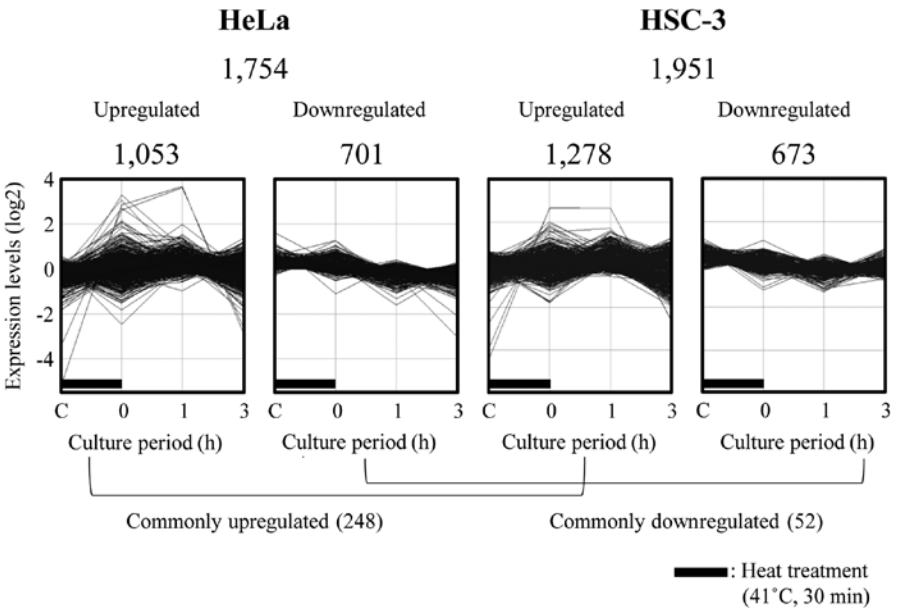

Figure 2. Global-scale gene expression analysis. HeLa and HSC-3 cells were incubated at 41˚C for 30 min and then cultured at 37˚C for 0-3 h. Gene expression analysis was performed using a GeneChip® microarray system and GeneSpring® software. The expression patterns of upregulated and downregulated probe sets in both cell lines are presented. Each number of probe sets is shown.

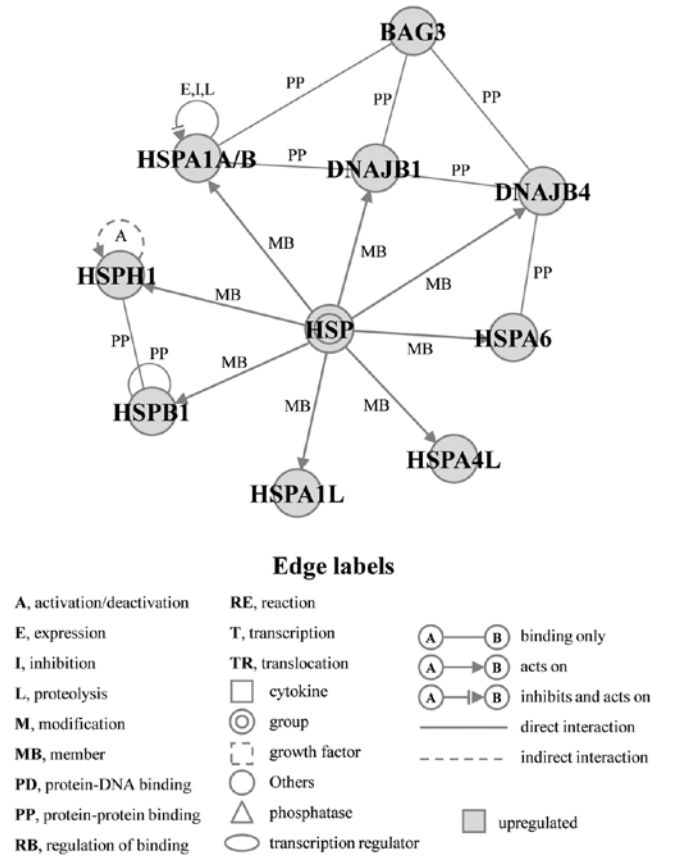

Figure 3. Gene network A. Commonly upregulated probe sets for the mild hyperthermia (MHT) conditions were analyzed by the Ingenuity Pathways Analysis software. The network is shown graphically as nodes (genes) and edges (the biological associations between the nodes).

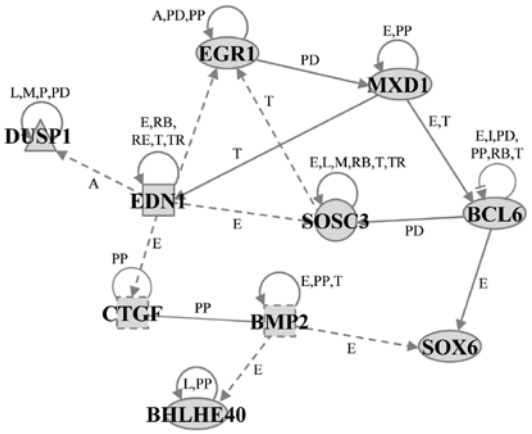

Figure 4. Gene network B. Commonly upregulated probe sets for the mild hyperthermia (MHT) conditions were analyzed by the Ingenuity Pathways Analysis software. The network is shown graphically as nodes (genes) and edges (the biological associations between the nodes). For edge labels, see Fig. 3.

(BHLHE40), BMP2, CTGF, dual specificity phosphatase 1 (DUSP1), EDN1, EGR1, MXD1, SOCS3 and SRY (sex determining region Y)-box 6 (SOX6) and was associated with the biological functions of cell death and survival (Fig. 4).

Verification of differentially expressed genes by real-time qPCR. To verify the alterations analyzed by the GeneChip microarray system, we used two cancer cell lines, MCF-7 and HO-1-N-1, in addition to the HeLa and HSC-3 cell lines. Time-dependent changes in the expression levels of selected genes that belonged to gene network A or B were measured using real-time qPCR. Among the genes in gene network A, the expression levels of HSPA1A/B, HSPA6, HSPH1, DNAJB1 and BAG3 were markedly elevated immediately following exposure to MHT in all four cell lines. The expression levels of BCL6, BMP2, CTGF, EDN1 and MXD1 in gene network B were significantly increased at 1 h following exposure to MHT in all four cell lines. The expression levels of EGR1 and SOCS3

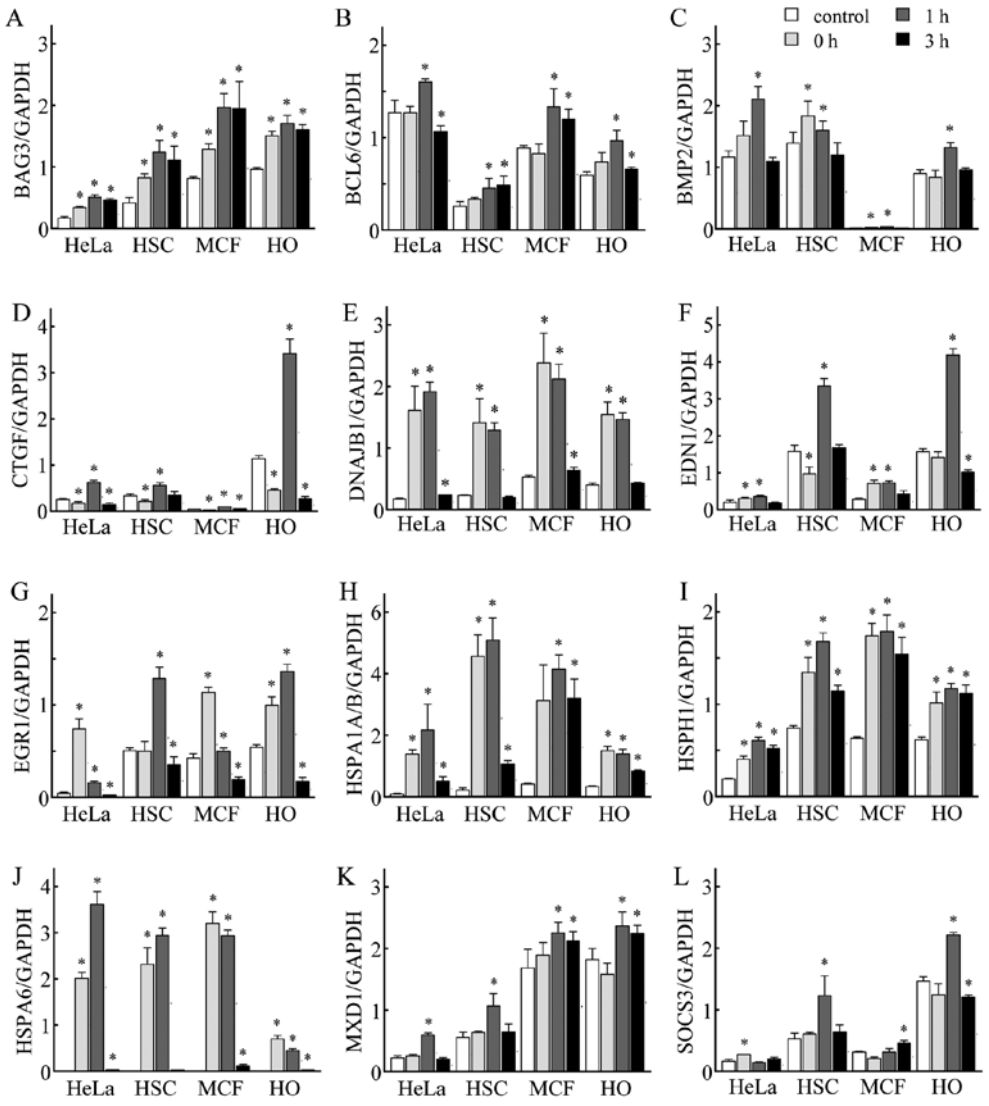

Figure 5. Verification of GeneChip® microarray results by real-time quantitative polymerase chain reaction (qPCR). The cancer cell lines, HeLa, HSC-3 (HSC), MCF-7 (MCF) and HO-1-N-1 (HO), were incubated at 41˚C for 30 min and then cultured at 37˚C for 0, 1 and 3 h. Real-time qPCR was performed. (A) BCL2-associated athanogene 3 (BAG3), (B) B-cell CLL/lymphoma 6 (BCL6), (C) bone morphogenetic protein 2 (BMP2), (D) connective tissue growth factor (CTGF), (E) DnaJ (Hsp40) homolog, subfamily B, member 1 (DNAJB1), (F) endothelin 1 (EDN1), (G) early growth response 1 (EGR1), (H) heat shock 70 kDa protein 1A/B (HSPA1A/B), (I) HSPH1, (J) heat shock 70 kDa protein 6 (HSP70B') (HSPA6), (K) MAX dimerization protein 1 (MXD1) and (L) suppressor of cytokine signaling 3 (SOCS3). Each expression level was normalized to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression level. Data are presented as the means ± SD (n=3-4). *P<0.05 vs. control (non-treated cells).

were significantly increased at 0, 1 and 3 h following exposure to MHT in all four cell lines. These results are in very close agreement with those of the GeneChip® analysis with the HeLa and HSC-3 cells (Fig. 5).

Discussion

HT is a well-established treatment modality that is used alone or in combination with radiotherapy and/or chemotherapy for the treatment of a variety of tumors (1). However, due to the complexity of the heat stress signal transduction, the elucidation of the molecular mechanisms involved in the cellular responses to heat stress has remained a major challenge in studies of HT. In this study, we used global-scale microarray analysis, in conjunction with bioinformatics tools, and identified unique common gene networks in response to MHT in cancer cells.

It is well known that a temperature of 42.5˚C for cancer cells is the inflection point of HT. That is, cancer cells exposed to temperatures >42.5˚C undergo cell death; however, the exposure of cells to a temperature of up to 42.5˚C induces little or no cytotoxicity (2). In the present study, the exposure of HeLa and HSC-3 human SCC cells to MHT at 41˚C, below the inflection point, did not affect cell viability or the cell cycle, as has also been shown in our previous studies (24-27). By contrast, several genes that were upregulated or downregulated by ≥1.5-fold were identified in both cancer cell lines following exposure to MHT. These results were comparable to those from previous studies on MHT conditions using cancer or normal cell lines (17,23-27). Previous studies have indicated that heat induces a significant decrease in mRNA and protein synthesis in order to increase the transcription of heat-responsive genes, such as HSPs, and that this overall reprogramming of gene expression permits the selective synthesis of HSPs (31,32).

Therefore, in this study, we focused on the common and upregulated genes elicited by MHT. We successfully identified two significant gene networks, A and B, derived from commonly upregulated genes in HeLa and HSC-3 cells exposed to MHT. In addition, the expression levels of 12 out of 19 genes selected were increased in the four cancer cell lines. Gene network A included several HSPs, as well as BAG3, and was specifically associated with the biological functions of cellular function and maintenance. Several microarray studies have demonstrated that the induction of HSPs is a common response to heat stress with or without cell death (15-27). HSPs protect cells both by limiting the effects of protein-damaging agents through protein chaperoning and refolding and by directly blocking the pathway of cell death, such as apoptosis or necrosis (12). In gene network A, HSPA1A/B (33,34), HSPB1 (35), HSPH1 (36) and DNAJB1 (33) were found to be associated with cytoprotection. BAG3 is a family of co-chaperons that interact with Hsp70 (37,38). The interaction between BAG3 and DNAJB1 or DNAJB4 has also been observed in human cells (39). The siRNA-mediated inhibition of BAG3 expression has been shown to increase apoptosis, indicating that BAG3 plays a role in the prevention of cell death (37,38).

Furthermore, Ingenuity Pathway Analysis allowed us to identify the significant gene network B, which was associated with the biological functions of cell death and survival. Seven genes in network B, BCL6 (40), BMP2 (41), CTGF (42), EDN1 (43), EGR1 (44), MXD1 (45) and SOCS3 (46), have been described as anti-cell death molecules in several types of cells, including cancer cells. Of note, we elucidated the interactions among the genes in gene network B: that is, EDN1 is involved in the upregulation of mRNAs for EGR1 (47), CTGF (48) and SOCS3 (49). It has also been reported that the binding of MXD1 DNA and EGR1 protein (50), as well as the protein-protein interaction between CTGF and BMP2 (51), occur in mammalian cells. Genes having cytoprotective or anti-cell death activity in gene networks A and B may play a role in protecting cancer cells from MHT stress.

Although HT is an important modality in cancer treatment, the acquisition of thermoresistance in cancer cells due to the elevation of HSPs makes HT less effective (13,14). The induction of HSPs is regulated mainly at the transcriptional level by the activation of heat shock transcription factor 1 (HSF1) (52,53). In addition, the induction of BAG3 is mediated, at least in part, by the activation of HSF1, as in the case of HSPs (54). Of note, the inhibition of the functions of HSF1 (55-57) or Hsp70 (58) by the use of gene targeting has been shown to sensitize HT-induced cell death in cancer cells. The targeting of heat shock response pathways, including these molecules in combination with HT may become a promising therapeutic approach for the treatment of cancer. In this study, we identified two gene networks comprising of several cytoprotective genes in four cancer cell lines exposed to MHT. Thus, the findings presented in this study may prove useful towards the design of more effective therapeutic strategies involving MHT or HT for the treatment of cancer.

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