Gene expression alterations of human liver cancer cells following borax exposure

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Abstract. Borax is a boron compound that is becoming widely recognized for its biological effects, including lipid peroxidation, cytotoxicity, genotoxicity, antioxidant activity and potential therapeutic benefits. However, it remains unknown whether exposure of human liver cancer (HepG2) cells to borax affects the gene expression of these cells. HepG2 cells were treated with 4 mM borax for either 2 or 24 h. Gene expression analysis was performed using Affymetrix GeneChip Human Gene 2.0 ST Arrays, which was followed by gene ontology analysis and pathway analysis. The clustering result was validated using reverse transcription-quantitative polymerase chain reaction. A cell proliferation assay was performed using Celigo Image Cytometer Instrumentation. Following this, 2- or 24-h exposure to borax significantly altered the expression level of a number of genes in HepG2 cells, specifically 530 genes (384 upregulated and 146 downregulated) or 1,763 genes (1,044 upregulated and 719 downregulated) compared with the control group, respectively (≥2-fold; P<0.05). Twenty downregulated genes were abundantly expressed in HepG2 cells under normal conditions. Furthermore, the growth of HepG2 cells was inhibited through the downregulation of PRUNE1, NBPF1, PPcaspase-1, UPF2 and MBTPS1 (≥1.5-fold, P<0.05).

Introduction

Boron is a naturally occurring element, representing 0.001% of the Earth's crust (1). Borax, which is also known as sodium tetraborate decahydrate (Na2B4O7·10H2O), is an important boron compound (2). In animals and humans, borax has been reported to be involved in metabolic processes associated with hormones and minerals (3). It has also been demonstrated to possess anti-inflammatory activity, indicating its therapeutic potential (4,5). Boron supplementation in the diet (borax, 100 mg/kg) has also been implicated to decrease lipid peroxidation and enhance antioxidant defense (6). Previous studies have suggested that the mechanism underlying the anti-inflammatory properties of borax involved the suppression of interleukin (caspase-)8, indicating that borax is potentially applicable for a bactericidal agent (7,8). However, numerous studies exploring the mutagenic properties of borax reported that its genotoxicity was nearly undetectable in bacteria and cultured mammalian cells (9,10). Furthermore, numerous studies exploring the mutagenic properties of borax reported that its genotoxicity was nearly undetectable in bacteria and cultured mammalian cells (9,10). Boron supplementation in the diet (borax, 100 mg/kg) has also been implicated to decrease lipid peroxidation and enhance antioxidant defense (6). Previous studies have suggested that the mechanism underlying the anti-inflammatory properties of borax involved the suppression of interleukin (caspase-)8, indicating that borax is potentially applicable for a bactericidal agent (7,8). However, numerous studies exploring the mutagenic properties of borax reported that its genotoxicity was nearly undetectable in bacteria and cultured mammalian cells (9,10). Furthermore, previous studies revealed that different concentrations of borax affected cell survival and cell growth in addition to altering the properties of a few chromosomes in humans, which were possibly caused by various genetic defects resulting from abnormalities in human chromosome (11,12). Additionally, borax has been widely known to have detrimental effects on lymphocyte proliferation, which is also highly vulnerable to induced sister chromatid exchange in human chromosomes (13). Thus, certain cellular toxicities indicated that those alterations were ascribed to genetic defects caused by borax in humans (14). Notably, it has been recently identified that borax treatment enhanced the resistance of DNA to titanium dioxide-induced...
damage (15). Taken together, numerous studies have focused on the application of borax for tumor prevention and demonstrated a strong inverse correlation between borax and various types of cancer, including prostate cancer, lung cancer, cervical cancer and hepatocellular carcinoma (HCC) (6-15). Although increasing studies have revealed various functions for borax, the underlying mechanisms of those effects remain unidentified, in particular regarding its genetic influences on various cells.

Our previous results indicated the effects of borax on tumor cells (HepG2) in vitro (Wu et al unpublished data). It was revealed that caspase--6 expression was increased following 2-h borax treatment in HepG2 cells and cell proliferation was inhibited following 24-h borax (4 mM) treatment. The numbers of living HepG2 cells and the borax concentrations were inversely correlated. Additionally, the 50% inhibitory concentration of borax was estimated as 4 mM (16). Although borax can be genotoxic at high doses, it is not highly mutagenic and does not easily form DNA adducts (17). Accordingly, borax is considered to induce oxidative stress through the depletion of glutathione and protein-bound sulfhydryl groups, which results in enhanced apoptosis and the production of reactive oxygen species (18,19). In brief, borax is predominately non-genotoxic and epigenetic mechanisms are likely to underlie the mechanism for its induction of carcinogenesis, during which the expression of multiple essential genes are altered (12).

Theoretically, exposure of HepG2 cells to borax for either 2 or 24 h may induce alterations in the expression levels in various critical genes, and these genes may therefore serve essential roles in various signaling pathways. The present study explored gene expression alterations directly caused by treatments with doses of borax (4 mM) in HepG2 cells for either 2 or 24 h and investigated the biological functions of those genes with significantly altered expression levels. Analysis of gene expression was performed through assessment of Affymetrix GeneChip data, followed by gene ontology (GO) analysis and pathway analysis.

Materials and methods

Cell culture. HepG2 cells were obtained from the China Center for Type Culture Collection (Wuhan University, Wuhan, China) and seeded in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS; cat. no. 10099-141; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) 1 day prior to borax (4 mM; Tianjin Bodi Chemical Co. Ltd., Tianjin, China) treatment in a humidified 5% CO2 incubator at 37°C for either 2 or 24 h. Following 2- or 24-h treatment with 4 mM borax, the culture medium was replenished with fresh media without borax.

RNA extraction and microarray hybridization. Following borax treatment, total RNA was extracted from HepG2 cells using TRIzol (cat. no. 3101-100; Invitrogen; Thermo Fisher Scientific, Inc.), followed by its purification using a miRNeasy Mini Kit (cat. no. 217004; Qiagen GmbH, Hilden, Germany). RNA integrity was also examined using an Agilent Bioanalyzer 2100 (grant no. G2938A; Agilent Technologies, Inc., Santa Clara, CA, USA). To obtain biotin-tagged cDNA, total RNA was subsequently amplified, labeled and purified using a WT PLUS Reagent kit (cat. no. 902280; Affymetrix; Thermo Fisher Scientific, Inc.). Array hybridization was performed using an Affymetrix GeneChip Human Gene 2.0 ST Array (Affymetrix; Thermo Fisher Scientific, Inc.) and Hybridization Oven 645 (cat. no. 60-0331-220V; Affymetrix; Thermo Fisher Scientific, Inc.), the Gene Chip was subsequently washed using a Hybridization, Wash and Stain Kit (cat. no. 900720; Affymetrix; Thermo Fisher Scientific, Inc.) in a Fluidics Station 450 (cat. no. 00-0079, Affymetrix; Thermo Fisher Scientific, Inc.). A GeneChip Scanner 3000 (cat. no. 00-00213; Affymetrix; Thermo Fisher Scientific, Inc.) was used to scan the results, which were controlled by Command Console Software 4.0 (Affymetrix; Thermo Fisher Scientific, Inc.) to summarize probe cell intensity data, namely, the CEL files with default settings. Following this, CEL files were normalized according to gene and exon level using Expression Console Software 4.0 (Affymetrix; Thermo Fisher Scientific, Inc.). All of the procedures, including array hybridization and scanning, were independently performed according to a standard protocol (20) for microarray experiments (n=3).

Validation of selected differentially expressed genes using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Single-stranded cDNAs were converted from 2.0 µg of total RNA extracted from cells using an RT kit (cat. no. M1701; Promega Corporation, Madison, WI, USA) with a temperature protocol of 72°C for 10 min. qPCR analysis was performed using 2.0 µg cDNA from each sample, pair-specific primers (Table I; Shanghai GeneChem Co., Ltd., Shanghai, China) and a SYBR green PCR Master Mix kit (cat. no. 639676; Takara Bio, Inc., Otsu, Japan). The thermocycling conditions used were as follows: 40 cycles at 95°C for 30 sec, 72°C for 45 sec, and 1 cycle at 72°C for 10 min. Quantitative measurement of the expression level of each gene was obtained by independent experiments (n=3). Samples were normalized to the expression level of GAPDH. Additionally, according to the ΔΔCq method (21), all of the results were detected as fold-change relative to the corresponding mRNA expression level in control cells.

Construction of adenoviral vectors. PCR was performed to amplify the encoding sequences of abundantly expressed genes. Gene interference RNA fragments (100 μmol; three codon sites; Table II) of those amplified sequences were subcloned into a plasmid (300 ng/μl; Shanghai GeneChem Co., Ltd., Shanghai, China) backbone using the T4DNA ligase (cat. no. 170702; Takara Bio, Inc.) following the digestion of the restriction enzyme. The pGCScaspase--404-iRNA and the GV115-NC were co-transformed into Escherichia coli GRM602 with backbone vector GV115-NC for homologous recombination. The recombinant plasmid pAd-iRNA digested with PacI (Fermentas; Thermo Fisher Scientific, Inc.) was used to transfect 293T cells (Thermo Fisher Scientific, Inc.) using Lipofectamine 2000 (cat. no. 11668-027; Invitrogen; Thermo Fisher Scientific, Inc.) for further packaging and amplification of the viruses and used in all groups (including any controls). The time interval was 72 h between transfection and subsequent experimentation. A control group (non-targeting shRNA) and positive control (specific-targeting shRNA) were used.
**Cell culture and transfection.** HepG2 cells were seeded in a 96-well black-bottom plate (1,500–2,500 cells/well; Corning Inc., Corning, NY, USA) filled with DMEM supplemented with FBS in a humidified incubator containing 5% CO\(_2\) at 37°C. The viral particles were added to serum-free medium when confluency reached 20–40%. The media was replaced with fresh medium supplemented with FBS following 12 h of incubation. Cells were subsequently incubated for a further 72 h until the transfection rate reached 70–90%. GFP expression was analyzed in HepG2 cells 48 and 72 h post-infection with AdGFP using fluorescence and light microscopy to determine the optimal transfection rate for subsequent experiments. Cells were subsequently collected for further use. Decreased expression of genes following treatment with shRNA was validated with RT-qPCR.

**Cell proliferation assay.** To identify the specific effects of those abundantly expressed genes on the proliferation of HepG2 cells, these cells were infected with adenovirus, seeded in a 96-well plate (2x10^3/well) and cultured in a humidified incubator containing 5% CO\(_2\) at 37°C for 24 h. The plates were scanned using Celigo Image Cytometer Instrumentation (Nexcelom Bioscience Instruments (Shanghai) Co., Ltd.m Shanghai, China) (22,23) to acquire images every 24 h, measuring the number of viable cells with 5-day sequential monitoring. Gross quantitative analyses were independently performed (n≥3), including the total number count, cell growth [shControl/experimental (transfected with RNAiMax) group, >1.5-fold change], position information and average integrated intensity of certain gated events for each fluorescence channel in individual wells.

**Statistical analysis.** A computational analysis of microarray data was performed using GeneSpring v12.0 (Agilent Technologies, Inc., Santa Clara, CA, USA). Based on a Student's t-test analysis, differentially expressed genes were filtered through statistical estimation of fold-changes from replicated samples (fold change ≥2.0) using a P-value threshold (P<0.05). Distinguishable gene expression of those samples was demonstrated via hierarchical clustering, followed by heatmap generation. Additionally, GO and pathway analyses of differentially expressed genes were performed to determine the potential signaling pathways underlying their biological functions. Public data from bioinformatics resources (http://www.geneontology.org/) were utilized for GO enrichment analysis. Ingenuity Pathway Analysis was utilized to identify genes whose expression was changed by at least 2-fold.

| Primer | Forward (5'-3') | Reverse (5'-3') | Length (bp) |
|--------|----------------|----------------|-------------|
| AZI2   | AACACTAAGGAATCGAATTCTG | GAGCAAAATGGGAGCAACAG | 186 |
| BPGM   | GCCTCTAAATGACGCTCACTAT | GGAGGCGGGTATACATTTG | 120 |
| FAM102B| TGCTGGTGAATCTGAATCTTTG | CTGGAGTTATTCTCCTGTCG | 236 |
| FBXO9  | AGTGGATGTTTGAACTTGCTC | GACCTGTTCTTCTGTTG | 121 |
| HOXB5  | GACCAAGTACCATGAACTTACGC | GCCACTGCAATATTTGAC | 120 |
| KIAA0430| ATATCCCTCTGGCTCCATGGCT | CTTTGGGAGTAAAGAAGGTT | 96 |
| MBTPS1 | TTTCATGCTCCCTGTTCTGTT | GCCCGATGCTGAGGGTTTAC | 280 |
| MYO10  | AGGGAGAAGTGGGAAGTGTG | CTTCTCCCTGAGGAACATCG | 192 |
| NBP1   | GCCCTGATGAGAAAGCATT | ATTCTTACGATCAGATGC | 146 |
| PRUN1  | GCTTCAGTACCCACCCCTAAC | AGAGGGCCTGCATCCCAAG | 278 |
| SETD5  | TAAGGCTGCTCTGGCTTCTT | CGCCTTCTGAGTTTGGTCTT | 246 |
| SNX13  | ATATCCCTCTGGCTCCATGGCT | CTTTGGGAGTAAAGAAGGTT | 281 |
| TSSR2  | CTTCTGCTTCTGCTTCTG | CTTTGGGAGTAAAGAAGGTT | 169 |
| TTB4L4 | CTTCCTCCTGCTGCTGCTG | AGAGGTCTGCTGCTGCTG | 154 |
| UPF2   | GGAGGATACCTAAGTGGCGAGTTTTT | GGTCGTTTGTGAGGTTT | 202 |
| RCN2   | TACAGGCTGAGTTCAGGTTGTCT | TCAAGGCCTGCAAGTTTAC | 252 |
| USP16  | AGAGGATACCTAAGTGGCGAGTTTTT | GGTCGTTTGTGAGGTTT | 236 |
| RASL11A| TATGCTGCTGCTGCTGCTG | CACGCATTGCGAGGATTGC | 120 |
| PPIP1  | CGCTGATGAGAAAGCATT | ATTCTTACGATCAGATGC | 291 |
| MTIF2  | TGGTTGCTGAAATGGGTTG | CACCGATGCTGAGGTTT | 276 |
| MAPK4  | CGGGTCTAATGCTGCTGCTG | GACGATGCTGAGGTTT | 151 |
| LMAN2L | ACTCGGTTCTGAGAAGCGCC | CTTGAGGATAGGGGATAT | 105 |
| CENPN  | TGAACACAGAACAACTCTGGAAGG | TCTGCTGCTGCTGCTG | 129 |
| CDC8A  | GAGCGAGGGAGGAATTTTACACA | TCTGGGAGAAGTACATTTGTCCT | 141 |
| EFR3A  | GCTGTCCCGCTTTTCGCTCCTC | AGAAGTGTCCTCAGTGCTCC | 232 |
| PPPIP5K2| ACTGGACAAACAGCGGTTGCTTAT | TGGGATATTTTGGTCACG | 167 |
Gene expression changes. Gene microarray analysis revealed that there were significant expression alterations of 530 genes in HepG2 cells in the 2-h borax treatment group compared with the control group (fold change ≥2.0; P<0.05). Among them, 146 genes were downregulated and 384 genes were upregulated (P<0.05; Fig. 1A). Furthermore, the expression levels of 1,763 genes were changed in HepG2 cells when the 24-h borax treatment group was compared with the control group (fold change ≥2.0; P<0.05). Among these genes, 719 were downregulated and 1,044 were upregulated (P<0.05; Fig. 1B).

Gene expression and GO analysis. Differentially expressed genes were stratified by treatment duration and presented as heatmaps either in red (upregulation) or green (downregulation), revealing an overall global change in expression for all genes (P<0.05; Fig. 2). Furthermore, detectable differences in gene expression patterns among those groups were also revealed by hierarchical clustering analyses. To determine the biological dysfunctionality associated with the altered gene expression induced by borax treatment, public data from bioinformatics resources (http://www.geneontology.org/) were utilized for GO enrichment analysis. Based on the cellular components, biological processes and molecular functions of each gene, significantly enriched GO terms were also arranged correspondingly (Fig. 3).

Pathway analysis. To determine which pathways were involved, Ingenuity Pathway Analysis was utilized to identify genes whose expression was changed by at least 2-fold. Furthermore, analyses of functional pathways indicated that the genes with expression levels that were significantly altered in cells from the 2-h treatment group compared with those in the control group were involved in seven KEGG pathways (P<0.01; Table III). Furthermore, significantly altered genes in cells from the 24-h treatment group compared with those in the control group were primarily associated with five KEGG pathways (P<0.01; Table IV).

Validation of the expression of genes by qPCR. To validate potentially valuable genes that were screened by microarray results, the results between the clustered selected transcripts and those from RT-qPCR were compared (Fig. 2). Following
borax treatment, 26 downregulated genes were identified on the basis of fold-change threshold, and the potentially functional correlation of caspase--6 or P53 signaling with proliferation in HepG2 cells was suggested. Additionally, RT-qPCR also revealed a few abundantly expressed genes, including AZI2, BPGM, FBXO9, MBTPS1, NBPF1, PRUNE1, SNX13, SETD5, TSR2, TTLL4, UPF2, USP16, PPCaspase-1, MTIF2, MAPK4, LMAN2L, CENPN, CDC8, and PPIPK2, in HepG2 cells with no borax treatment.

Effects of abundantly expressed genes on cell proliferation. HepG2 cells infected with recombinant adenovirus were cultured for 48-72 h. When adenoviral green fluorescent protein (AdGFP) reached over 80%, recombinant adenovirus was considered to be efficiently infected HepG2 cells in vitro (Fig. 4), and decreased expression of genes was established following transfection with each shRNA (Fig. 5). On the 5th day following the infection, the proliferation of iRNA-treated HepG2 cells was significantly suppressed (fold change ≥1.5) compared with those in the control group (P<0.05). Furthermore, the target genes of RNAi fragments included PRUNE1, NBPF1, PPCaspase-1, UPF2, and MBTPS1 (fold change ≥1.50; Fig. 6). These findings indicated that, compared with control group cells, cell proliferation in the shRNA group was significantly reduced (fold change ≥1.5). Therefore, it was inferred that the target gene of RNA lentivirus in the shRNA group was tumor cells proliferation-related positive gene.
Discussion

Boron is a naturally abundant element on the earth (24). Notably, borax is a boron compound, which plays essential roles in many industries and in daily life (25). Currently, several boron-containing molecules have been applied for the treatment of multiple diseases, including inflammation, diabetes and cancer (26,27). Some of these treatments have produced positive results in preclinical and clinical trials (28,29). For instance, previous studies revealed boric acid/borax mediated protection against TiO\(_2\) genotoxicity in peripheral blood cells (30). In addition, borax mediated the stimulation of sister chromatid exchange in human chromosomes and/or lymphocyte proliferation (1). Furthermore, a previous study revealed that peripheral blood cells with aflatoxin B1-induced genetic damage were sufficiently rescued by borax treatment, which has also been indicated to be an effective antiepileptic drug (31,32).

The properties of borax are also considered to be correlated with genetic defects and genotoxicity. Specifically, it is widely accepted that when borax is applied at high concentrations, it is cytotoxic to mammalian cells, although cell transformation assays show that borax treatment is weakly mutagenic and not oncogenic (33). In our previous study, it was indicated that borax induced a strong increase in caspase--6 production, which was accompanied by the enhanced expression of p53-modulated genes, including p21, Bax and Puma (16). Considering that the precise regulation of borax-induced genotoxicity has not been well defined, novel mechanisms underlying the genetic actions and potential new biological effects of borax on various cell-types require more insight.

In the present study, microarray analysis indicated that the expression levels of 530 genes were changed in HepG2 cells in the 2-h treatment group. Among them, 146 were downregulated and 384 were upregulated. Notably, MYO10, one of the downregulated genes, encodes a member of the myosin superfamily, which mediates the migration and invasion of tumor cells, suggesting that it contributes to the metastatic phenotype, possibly via its direct involvement in the assembly of molecular motors (34,35). miR-4521 was also downregulated, which is closely correlated with signal transduction, mediating DNA binding, receptor activity and other processes (36). The DDIT3 gene, which encodes a suppressor protein that primarily inhibits mTOR signaling under stress conditions...
Table III. Differentially expressed genes involved in signal transduction (2 h vs. Control group).

| Pathway/ genebank ID | Probe_Set_ID | Gene symbol | Description of expression product | Fold change | P-values | Regulation after borax treatment |
|----------------------|--------------|-------------|-----------------------------------|-------------|---------|----------------------------------|
| hsa04010:MAPK signaling pathway | | | | | | |
| NM_001202233 | TC12000414.hg.1 | NR4A1 | Nuclear receptor subfamily 4, group A, member 1 | 21.7 | 0.000881 | Up |
| NM_005252 | TC11001948.hg.1 | FOS | FBJ murine osteosarcoma viral oncogene homolog | 11.3 | 0.000164 | Up |
| NM_001199741 | TC01000745.hg.1 | GADD45A | Growth arrest and DNA-damage-inducible, alpha | 10.7 | 0.000054 | Up |
| NM_004419 | TC10000801.hg.1 | DUSP5 | Dual specificity phosphatase 5 | 10.6 | 0.000096 | Up |
| NM_000575 | TC02002218.hg.1 | IL1A | Interleukin-1, alpha | 8.3 | 0.005040 | Up |
| NM_001394 | TC08001099.hg.1 | DUSP4 | Dual specificity phosphatase 4 | 5.6 | 0.002509 | Up |
| NM_005354 | TC19001285.hg.1 | JUND | Jun D proto-oncogene | 3.4 | 0.001100 | Up |
| NM_015675 | TC19000055.hg.1 | GADD45B | Growth arrest and DNA-damage-inducible, beta | 3.3 | 0.000382 | Up |
| NM_001195053 | TC12001625.hg.1 | DDIT3 | DNA-damage-inducible transcript 3 | 2.3 | 0.005761 | Up |
| NM_030640 | TC12001255.hg.1 | DUSP16 | Dual specificity phosphatase 16 | 2.3 | 0.000079 | Up |
| NM_000576 | TC02002219.hg.1 | IL1B | Interleukin-1, beta | 2.1 | 0.016505 | Up |
| NM_004651 | TC05001184.hg.1 | MYO10 | Myosin 10 | -7.17 | 0.001269 | Down |
| NM_005345 | TC06000384.hg.1 | HSPA1A | Heat shock 70 kDa protein 1A | -4.2 | 0.011012 | Down |
| NM_005346 | TC06000385.hg.1 | HSPA1B | Heat shock 70 kDa protein 1B | -4.3 | 0.010610 | Down |
| NM_002228 | TC01001927.hg.1 | JUN | Jun proto-oncogene | -2.1 | 0.000287 | Down |
| hsa04064:NF-kappa B signaling pathway | | | | | | |
| NM_009963 | TC01003638.hg.1 | PTGS2 | Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) | 75.7 | 0.000000 | Up |
| NM_006290 | TC06001027.hg.1 | TNFAIP3 | Tumor necrosis factor, alpha-induced protein 3 | 68.3 | 0.000000 | Up |
| NM_020529 | TC14001036.hg.1 | NFKBIA | Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha | 9.2 | 0.000014 | Up |
| NM_001165 | TC11000956.hg.1 | BIRC3 | Baculoviral IAP repeat containing 3 | 3.7 | 0.000004 | Up |
| NM_002089 | TC04001286.hg.1 | CXCL2 | Chemokine (C-X-C motif) ligand 2 | 4.0 | 0.010873 | Up |
| NM_015675 | TC19000055.hg.1 | GADD45B | Growth arrest and DNA-damage-inducible, beta | 3.3 | 0.000382 | Up |
| NM_000576 | TC02002219.hg.1 | IL1B | Interleukin-1, beta | 2.1 | 0.016505 | Up |
| hsa04621:NOD-like receptor signaling pathway | | | | | | |
| NM_006290 | TC06001027.hg.1 | TNFAIP3 | Tumor necrosis factor, alpha-induced protein 3 | 68.3 | 0.000000 | Up |
| NM_020529 | TC14001036.hg.1 | NFKBIA | Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha | 9.2 | 0.000014 | Up |
| NM_002089 | TC04001286.hg.1 | CXCL2 | Chemokine (C-X-C motif) ligand 2 | 4.0 | 0.010873 | Up |
| NM_001165 | TC11000956.hg.1 | BIRC3 | Baculoviral IAP repeat containing 3 | 3.7 | 0.000004 | Up |
Table III. Continued.

| Pathway/ genebank ID | Probe_Set_ID            | Gene Symbol | Description of expression product                                                                 | Fold change | P-values   | Regulation after borax treatment |
|----------------------|-------------------------|-------------|--------------------------------------------------------------------------------------------------|-------------|------------|----------------------------------|
| NM_000576            | TC02002219.hg.1         | IL1B        | Interleukin-1, beta                                                                               | 2.1         | 0.016505   | Up                               |
| NM_000600            | TC05002383.hg.1         | IL6         | Interleukin-6                                                                                    | 2.4         | 0.007231   | Up                               |
| NM_100616406         | TC17000132.hg.1         | MIR4521     | MicroRNA 4521                                                                                    | -6.61       | 0.000125   | Down                             |

hsa04115:53 signaling pathway

| NM_001199741         | TC01000745.hg.1         | GADD45A     | Growth arrest and DNA-damage-inducible, alpha                                                     | 10.7        | 0.000054   | Up                               |
| NM_003246            | TC15000270.hg.1         | THBS1       | Thrombospondin 1                                                                                 | 6.5         | 0.002300   | Up                               |
| NM_000602            | TC07000643.hg.1         | SERPINE1    | Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1    | 4.7         | 0.010348   | Up                               |
| NM_021127            | TC18000213.hg.1         | PMAIP1      | Phorbol-12-myristate-13-acetate-induced protein 1                                                 | 4.7         | 0.000034   | Up                               |
| NM_015675            | TC19000055.hg.1         | GADD45B     | Growth arrest and DNA-damage-inducible, beta                                                     | 3.3         | 0.000382   | Up                               |

hsa04141: Protein processing in endoplasmic reticulum

| NM_014330            | TC19000711.hg.1         | PPP1R15A    | Protein phosphatase 1, regulatory subunit 15A                                                     | 4.4         | 0.006746   | Up                               |
| NM_018566            | TC01003773.hg.1         | YOD1        | YOD1 OTU deubiquinating enzyme 1 homolog                                                         | 3.7         | 0.000181   | Up                               |
| NM_001433            | TC17001796.hg.1         | ERN1        | Endoplasmic reticulum to nucleus signaling 1                                                      | 2.6         | 0.000008   | Up                               |
| NM_001195053         | TC12001625.hg.1         | DDIT3       | DNA-damage-inducible transcript 3                                                                | 2.3         | 0.005761   | Up                               |
| NM_005346            | TC06000385.hg.1         | HSPA1B      | Heat shock 70 kDa protein 1B                                                                      | -4.3        | 0.010610   | Down                             |
| NM_005345            | TC06000384.hg.1         | HSPA1A      | Heat shock 70 kDa protein 1A                                                                      | -4.2        | 0.011012   | Down                             |
| NM_003791            | TC16001307.hg.1         | MBTPS1      | Membrane-bound transcription factor peptidase, site 1                                             | -3.2        | 0.000643   | Down                             |
| NM_001172415         | TC09001009.hg.1         | BAG1        | BCL2-associated athanogene                                                                       | -2.1        | 0.000440   | Down                             |

hsa04668: TNF signaling pathway

| NM_000963            | TC01003638.hg.1         | PTGS2       | Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)            | 75.7        | 0.000000   | Up                               |
| NM_006290            | TC06001027.hg.1         | TNFAIP3     | Tumor necrosis factor, alpha-induced protein 3                                                    | 68.3        | 0.000000   | Up                               |
| NM_001168319         | TC06000087.hg.1         | EDN1        | Endothelin 1                                                                                     | 13.1        | 0.000004   | Up                               |
| NM_005252            | TC11001948.hg.1         | FOS         | FBJ murine osteosarcoma viral oncogene homolog                                                    | 11.3        | 0.000164   | Up                               |
| NM_020529            | TC14001036.hg.1         | NFKB1A      | Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha                | 9.2         | 0.000014   | Up                               |
| NM_002089            | TC04001286.hg.1         | CXCL2       | Chemokine (C-X-C motif) ligand 2                                                                  | 4.0         | 0.010873   | Up                               |
| NM_001165            | TC11000956.hg.1         | BIRC3       | Baculoviral IAP repeat containing 3                                                                | 3.7         | 0.000004   | Up                               |
| NM_001130046         | TC02001364.hg.1         | CCL20       | Chemokine (C-C motif) ligand 2                                                                    | 3.0         | 0.002008   | Up                               |
and is partially involved in cancer progression (37), was also downregulated with borax treatment. Heat shock protein (HSP)25 protein is encoded by the HSP β‑1 gene. HSPβ‑1 is a member of the HSP family (38) and is abundantly expressed in various types of cancer associated with poor prognosis and resistance to chemotherapy, possibly through their aggressive tumor behavior and metastasis (39). In the present study, HSPβ‑1 was also significantly downregulated. Early growth response protein 1, which is involved in the initial stage of the inflammatory response, possibly through its critical roles as a tumor suppressor or promoter (40), was upregulated following 2‑h borax treatment in the present study. Furthermore, prostaglandin-endoperoxide synthase 2, a principal inflammatory mediator and a UV‑inducible enzyme the catalyzes the first step in the synthesis of prostaglandin E2 (41), was also upregulated. Additionally, TNFAIP3 and caspase‑6, which are associated with inflammation and stress reaction (42), were also found to be upregulated. Notably, TNFAIP3 acts as a critical molecular switch to discriminate tumor necrosis factor‑induced NF‑κB signaling from the activated JNK signaling pathways in hepatocytes when stimulated with varying cytokine concentrations under normal or pathological conditions (43). These findings implicate downregulated/upregulated genes following borax treatment impact the migration and invasion of tumor cells, DNA binding signal transduction, inflammation and stress reactions. However, the specific mechanisms involved require further study.

The expression levels of 1,763 genes were changed in cells from the 24-h treatment group compared with those in the control group. Specifically, 719 genes were downregulated and 1,044 genes were upregulated (Fig. 1). Among them, the downregulated genes included B3GALT6, a critical enzyme catalyzing the formation of the tetrasaccharide linkage region, the mutation of which results in proteoglycan maturation defects (44). In the present study, FAM20B was downregulated in the 24‑h treatment group. Notably, it was previously indicated that FAM20B deletion is associated with Ehlers‑Danlos syndrome (45,46). UBE4B was also downregulated in cells from the 24-h treatment group in the present study. A previous study revealed that silencing of UBE4B expression inhibited the proliferation, colony formation, migration and invasion of liver cancer cells in vitro, and resulted in significant apoptosis. Therefore, it was suggested that this gene may be a good prognostic candidate for liver cancer (47). The overexpression of UBE4B, which is

| Pathway/ genebank ID | Probe_Set_ID | Gene symbol | Description of expression product | Fold change | P‑values | Regulation after borax treatment |
|----------------------|--------------|-------------|-----------------------------------|-------------|----------|---------------------------------|
| NM_000600            | TC05002383.hg.1 IL6  | Interleukin‑6       | 2.4  | 0.007231  | Up       |
| NM_000576            | TC02002219.hg.1 IL1B | Interleukin‑1, beta | 2.1  | 0.016505  | Up       |
| NM_003955            | TC17001917.hg.1 SOCS3 | Suppressor of cytokine signaling 3 | 2.1  | 0.003726  | Up       |
| NM_002228            | TC01001927.hg.1 JUN  | Jun proto‑oncogene | -2.1 | 0.000287  | Down     |
| NM_005252            | TC11001948.hg.1 FOS  | FBJ murine osteosarcoma viral oncogene homolog | 11.3 | 0.000164  | Up       |
| NM_020529            | TC14001036.hg.1 NFKBIA | Nuclear factor of kappa light polypeptide gene enhancer in B‑cells inhibitor, alpha | 9.2  | 0.000014  | Up       |
| NM_000600            | TC05002383.hg.1 IL6  | Interleukin‑6       | 2.4  | 0.007231  | Up       |
| NM_000576            | TC02002219.hg.1 IL1B | Interleukin‑1, beta (IL1B) | 2.1  | 0.016505  | Up       |
| NM_002228            | TC01001927.hg.1 JUN  | Jun proto‑oncogene | -2.1 | 0.000287  | Down     |

hsa04620:Toll‑like receptor signaling pathway

| Pathway/ genebank ID | Probe_Set_ID | Gene symbol | Description of expression product | Fold change | P‑values | Regulation after borax treatment |
|----------------------|--------------|-------------|-----------------------------------|-------------|----------|---------------------------------|
| NM_005252            | TC11001948.hg.1 FOS  | FBJ murine osteosarcoma viral oncogene homolog | 11.3 | 0.000164  | Up       |
| NM_020529            | TC14001036.hg.1 NFKBIA | Nuclear factor of kappa light polypeptide gene enhancer in B‑cells inhibitor, alpha | 9.2  | 0.000014  | Up       |
| NM_000600            | TC05002383.hg.1 IL6  | Interleukin‑6       | 2.4  | 0.007231  | Up       |
| NM_000576            | TC02002219.hg.1 IL1B | Interleukin‑1, beta (IL1B) | 2.1  | 0.016505  | Up       |
| NM_002228            | TC01001927.hg.1 JUN  | Jun proto‑oncogene | -2.1 | 0.000287  | Down     |

Figure 4. Efficiency of adenovirus infection in HepG2 cells. GFP expression was analyzed in HepG2 cells 48 and 72 h post‑infection with AdGFP using fluorescence (lower panels) and light (phase‑contrast; upper panels) microscopy (magnification, x100) to determine the optimal transfection rate for subsequent experiments. (A) (48 h) 40% and (B) (72 h) 80% of cells exhibited GFP expression, respectively. AdGFP, adenoviral green fluorescent protein.
Table IV. Differentially expressed genes involved in signal transduction (24 h vs. control group).

| Pathway/ Genebank ID | Probe_Set_ID | Gene symbol | Description of expression product | Fold change | P-values | Regulation after borax treatment |
|----------------------|--------------|-------------|-----------------------------------|-------------|----------|----------------------------------|
| hsa04110:Cell cycle  |              |             |                                   |             |          |                                  |
| NM_002392            | TC12000606.hg.1 | MDM2       | Mdm2, p53 E3 ubiquitin protein ligase homolog | 13.8        | 0.00010  | Up                               |
| NM_001199741         | TC01000745.hg.1 | GADD45A    | Growth arrest and DNA-damage-inducible, alpha | 7.9         | 0.00006  | Up                               |
| NM_000389             | TC06000532.hg.1 | CDKN1A     | Cyclin-dependent kinase inhibitor 1A (p21, Cip1) | 4.4         | 0.00015  | Up                               |
| NM_001259             | TC07001603.hg.1 | CDK6       | Cyclin-dependent kinase 6          | 4.3         | 0.00013  | Up                               |
| NM_001079846          | TC16000823.hg.1 | CREBBP     | CREB binding protein (CREBBP)      | 3.0         | 0.00007  | Up                               |
| NM_001799             | TC05000301.hg.1 | CDK7       | Cyclin-dependent kinase 7          | 2.8         | 0.00039  | Up                               |
| NM_007637             | TC1001228.hg.1 | ZNF84      | Zinc finger protein 84             | 2.42        | 0.00063  | Up                               |
| NM_001789             | TC03001374.hg.1 | CDC25A     | Cell division cycle 25 homolog A  | 2.4         | 0.00041  | Up                               |
| NM_002553             | TC07001724.hg.1 | ORC5       | Origin recognition complex, subunit 5 | 2.3         | 0.00012  | Up                               |
| BC012827              | TC01000545.hg.1 | CDC20      | Cell division cycle 20 homolog    | 2.2         | 0.00364  | Up                               |
| NM126792              | TC05001184.hg.1 | B3GALT6    | Beta 1,3-galactosyltransferase polypeptide 6 | -18.97      | 0.00000  | Down                             |
| NM009917              | TC06001313.hg.1 | FAM20B     | Family with sequence similarity 20, member B | -5.13       | 0.00002  | Down                             |
| NM_001262             | TC01000619.hg.1 | CDKN2C     | Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4) | -4.8        | 0.00364  | Down                             |
| NM_003318             | TC06000761.hg.1 | TTK        | TTK protein kinase                 | -3.7        | 0.00008  | Down                             |
| NM_001237             | TC04001516.hg.1 | CCNA2      | Cyclin A2 (CCNA2)                  | -3.5        | 0.00007  | Down                             |
| NM_004701             | TC15000449.hg.1 | CCNB2      | Cyclin B2 (CCNB2)                  | -2.7        | 0.00011  | Down                             |
| NM_005611             | TC16000448.hg.1 | RBL2       | Retinoblastoma-like 2 (p130)       | -2.6        | 0.00001  | Down                             |
| NM_001178138          | TC03001849.hg.1 | TFDP2      | Transcription factor Dp-2 (E2F dimerization partner 2) | -2.5        | 0.00001  | Down                             |
| NM_001786             | TC02001182.hg.1 | CDK1       | Cyclin-dependent kinase 1          | -2.5        | 0.00163  | Down                             |
| NM_002388             | TC06001799.hg.1 | MCM3       | Minichromosome maintenance complex component 3 | -2.5        | 0.00000  | Down                             |
| NM_057749             | TC08001438.hg.1 | CCNE2      | Cyclin E2 (CCNE2)                  | -2.4        | 0.00622  | Down                             |
| NM_001042749          | TC0X000066.hg.1 | STAG2      | Stromal antigen 2 (STAG2)          | -2.2        | 0.00017  | Down                             |
| NM_005915             | TC02002376.hg.1 | MCM6       | Minichromosome maintenance complex component 6 | -2.1        | 0.00019  | Down                             |
| NM_001136197          | TC19000070.hg.1 | FZR1       | Fizzy/cell division cycle 20 related 1 | -2.1        | 0.00348  | Down                             |
| NM_022809             | TC05001829.hg.1 | CDC25C     | Cell division cycle 25 homolog C   | -2.1        | 0.00092  | Down                             |

hsa04115:p53 signaling pathway

| Pathway/ Genebank ID | Probe_Set_ID | Gene symbol | Description of expression product | Fold change | P-values | Regulation after borax treatment |
|----------------------|--------------|-------------|-----------------------------------|-------------|----------|----------------------------------|
| NM_002392            | TC12000606.hg.1 | MDM2       | Mdm2, p53 E3 ubiquitin protein ligase homolog | 13.8        | 0.00010  | Up                               |
| NM_008870             | TC13000386.hg.1 | IER3       | Immediate early response 3        | 8.47        | 0.00200  | Up                               |
| NM_001199741          | TC01000745.hg.1 | GADD45A    | Growth arrest and DNA-damage-inducible, alpha | 7.9         | 0.00006  | Up                               |
| NM_021127             | TC18000213.hg.1 | PMAIP1     | Phorbol-12-myristate-13-acetate-induced protein 1 | 6.1         | 0.00001  | Up                               |
| NM_000389             | TC06000532.hg.1 | CDKN1A     | Cyclin-dependent kinase inhibitor 1A | 4.4         | 0.00015  | Up                               |
| NM_001259             | TC07001603.hg.1 | CDK6       | Cyclin-dependent kinase 6          | 4.3         | 0.00013  | Up                               |
| NM_001172477          | TC08001496.hg.1 | RRM2B      | Ribonucleotide reductase M2 B      | 3.7         | 0.00015  | Up                               |
| NM_001199933          | TC06001997.hg.1 | SESN1      | Sestrin 1                          | 3.6         | 0.00004  | Up                               |
| NM_000602             | TC07000643.hg.1 | SERPINE1   | Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 | 2.2         | 0.00406  | Up                               |
Table IV. Continued.

| Pathway/Genebank ID | Probe_Set_ID | Gene symbol | Description of expression product | Fold change | P-values | Regulation after borax treatment |
|---------------------|--------------|-------------|-----------------------------------|-------------|---------|----------------------------------|
| NM_004324           | TC19000716.hg.1 | BAX         | BCL2-associated X protein          | 2.2         | 0.00672 | Up                               |
| NM_001034           | TC02000557.hg.1 | RRM2        | ribonucleotide reductase M2        | -2.9        | 0.00010 | Down                             |
| NM_002639           | TC18000226.hg.1 | SERPINB5    | Serpin peptidase inhibitor, clade B (ovalbumin), member 5 | -2.8 | 0.00756 | Down                             |
| NM_001196           | TC01000866.hg.1 | BID         | BH3 interacting domain death agonist | -2.5 | 0.00002 | Down                             |
| NM_016426           | TC22000394.hg.1 | GTSE1       | G-2 and S-phase expressed 1       | -2.4        | 0.00019 | Down                             |
| NM_003620           | TC17000739.hg.1 | PPM1D       | Protein phosphatase, Mg2+/Mn2+ dependent, 1D | 4.5 | 0.00005 | Up                               |
| NM_003842           | TC08001049.hg.1 | TNFRSF10B   | Tumor necrosis factor receptor superfamily, member 10b | 3.8 | 0.00005 | Up                               |
| NM_031459           | TC01000377.hg.1 | SESN2       | Sestrin 2                         | 3.7         | 0.00119 | Up                               |
| NM_003246           | TC15000270.hg.1 | THBS1       | Thrombospondin 1                  | 2.5         | 0.00013 | Up                               |
| NM_010277           | TC66000070.hg.1 | UBE4B       | Ubiquitination factor E4B         | -17.44      | 0.00000 | Down                             |
| NM_005351           | TC62000079.hg.1 | PLOD1       | Procollagen-lysine, 2-oxoglutarate 5-dioxxygenase 1 | -16.78 | 0.00104 | Down                             |
| NM_004701           | TC15000449.hg.1 | CCNB2       | Cyclin B2                         | -2.7        | 0.00011 | Down                             |
| NM_001786           | TC02001182.hg.1 | CDK1        | Cyclin-dependent kinase 1         | -2.5        | 0.00163 | Down                             |
| NM_057749           | TC08001438.hg.1 | CCNE2       | Cyclin E2                         | -2.4        | 0.00622 | Down                             |
| NM_022470           | TC03002022.hg.1 | ZMAT3       | Zinc finger, matrin-type 3        | -2.2        | 0.00161 | Down                             |

hsa04668:TNF signaling pathway

| Pathway/Genebank ID | Probe_Set_ID | Gene symbol | Description of expression product | Fold change | P-values | Regulation after borax treatment |
|---------------------|--------------|-------------|-----------------------------------|-------------|---------|----------------------------------|
| NM_006290           | TC06001027.hg.1 | TNFAIP3     | Tumor necrosis factor, alpha-induced protein 3 | 20.2 | 0.00007 | Up                               |
| NM_006941           | TC11001948.hg.1 | TCF19       | Transcription factor 19           | 8.96        | 0.00064 | Up                               |
| NM_001168319        | TC06000087.hg.1 | EDN1        | Endothelin 1                      | 3.0         | 0.00025 | Up                               |
| NM_001145138        | TC11001939.hg.1 | RELA        | V-rel reticuloendotheliosis viral oncogene homolog A (avian) | 3.0 | 0.00002 | Up                               |
| NM_001244134        | TC10002935.hg.1 | MAP3K8      | Mitogen-activated protein kinase kinase kinase 8 | 2.9 | 0.00013 | Up                               |
| NM_000214           | TC20000621.hg.1 | JAG1        | Jagged 1                          | 2.7         | 0.00019 | Up                               |
| NM_000963           | TC01003638.hg.1 | PTGS2       | Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) | 2.6 | 0.02677 | Up                               |
| NM_001166           | TC11000957.hg.1 | BIRC2       | Baculoviral IAP repeat containing 2 | 2.3         | 0.00069 | Up                               |
| NM_000600           | TC05001366.hg.1 | IL6         | Interleukin-6                     | 2.2         | 0.00002 | Up                               |
| NM_182810           | TC22000317.hg.1 | ATF4        | Activating transcription factor 4 (tax-responsive enhancer element B67) | 2.2 | 0.00054 | Up                               |
| NM_029914           | TC61000040.hg.1 | UBIAD1      | UbiA prenyltransferase domain containing 1 | -16.88 | 0.00108 | Down                             |
| NM_001256045        | TC03001824.hg.1 | PIK3CB      | Phosphoinositide-3-kinase, catalytic, beta polypeptide | -4.7 | 0.00000 | Down                             |
| NM_001065           | TC12001135.hg.1 | TNFRSF1A    | Tumor necrosis factor receptor superfamily, member 1A | -4.0 | 0.00035 | Down                             |
| NM_002758           | TC17000807.hg.1 | MAP2K6      | Mitogen-activated protein kinase kinase 6 | -4.0 | 0.00021 | Down                             |
| NM_002982           | TC17000383.hg.1 | CCL2        | Chemokine (C-C motif) ligand 2 | -2.6 | 0.03428 | Down                             |
| NM_00114172         | TC01002616.hg.1 | PIK3R3      | Phosphoinositide-3-kinase, regulatory subunit 3 (gamma) | -2.3 | 0.00083 | Down                             |
| NM_005027           | TC19002628.hg.1 | PIK3R2      | Phosphoinositide-3-kinase, regulatory subunit 2 (beta) | -2.3 | 0.00044 | Down                             |
widely accepted as a p53 upstream target gene, contributes to the migration and invasion of tumor cells (48,49). UBIAD1, also known as UbiA prenyltransferase domain-containing protein 1, functions as an important regulator in the cell progression of bladder and prostate cancer, as well as vascular integrity, possibly through its modulation of metabolism of intracellular

| Pathway/ Genebank ID | Probe_Set_ID | Gene symbol | Description of expression product | Fold change | P-values | Regulation after borax treatment |
|----------------------|--------------|-------------|-----------------------------------|-------------|----------|----------------------------------|
| NM_001136153, NM_001199427 | TC06004121.hg.1, TC14000786.hg.1 | ATF6B, TRAF3 | Activating transcription factor 6 beta, TNF receptor-associated factor 3 (TRAF3) | -2.1, -2.1 | 0.00045, 0.00073 | Down, Down |

hsa04512:AMPK signaling pathway

| Pathway/ Genebank ID | Probe_Set_ID | Gene symbol | Description of expression product | Fold change | P-values | Regulation after borax treatment |
|----------------------|--------------|-------------|-----------------------------------|-------------|----------|----------------------------------|
| NM_003749, NM_000875, NM_181715 | TC13000871.hg.1, TC15000949.hg.1, TC01003280.hg.1 | IRS2, IGFlR, CRTC2 | Insulin receptor substrate 2, Insulin-like growth factor 1 receptor, CREB regulated transcription coactivator 2 | 3.5, 3.5, 3.0 | 0.00044, 0.00188, 0.00176 | Up, Up, Up |

hsa04621:NOD-like receptor signaling pathway

| Pathway/ Genebank ID | Probe_Set_ID | Gene symbol | Description of expression product | Fold change | P-values | Regulation after borax treatment |
|----------------------|--------------|-------------|-----------------------------------|-------------|----------|----------------------------------|
| NM_006253 | TC12000936.hg.1 | PRKAB1 | Protein kinase, AMP-activated, beta 1 non-catalytic subunit | 2.7 | 0.00031 | Up |

Table IV. Continued.
cholesterol and protection against oxidative stress (50). UBIAD1 was also downregulated. Additionally, PLOD1, which is associated with cell apoptosis, cell cycle and metastasis (51), was also found to be downregulated.

In the present study, 24-h treatment with borax upregulated the expression of several genes, including ZNF84, which is also known as a zinc finger transcription factor gene (52). ZNF84 is located in chromosome 12q24.33, which is correlated with recurrent breakpoints and allelic loss in a few types of cancer (52,53). Immediate early response 3 was another upregulated gene that normally regulates apoptosis, proliferation and the maintenance of HCCs (54,55). TCF19, which was also upregulated, has been identified to be a good prognostic candidate for HCC, thereby becoming a promising candidate for preclinical and/or clinical studies to determine its potential risk in HCCs (56).

Distinct sets of genes were found to be altered after different treatment durations, namely, borax treatments for either 2 or 24 h in HepG2 cells. Exposure to borax for 2 h altered the expression levels of genes encoding proteins involved in signal transduction underlying stress response, biopolymer metabolic process, the inflammatory response (e.g., NF-κB and caspase-6) and unfolded protein response among other possibilities. Notably, the results for cells from the 2-h treatment group revealed the disruption of certain metabolic processes involved in inflammation and stress response. Accordingly, borax treatment for 24 h caused the dysregulation of genes involved in a number of signaling pathways, which are associated with enhanced cell proliferation and apoptosis underlying the disruption of both vascular integrity and suppression of tumor cell progression (16), indicating that the disruption of those signaling pathways may contribute to carcinogenesis in borax-treated HepG2 cells.

Enriched GO analyses in the present study revealed that the significantly enriched gene sets included the response to primary metabolic process, response to stimulus, biosynthetic process, developmental process, apoptotic process, immune system process, binding, catalytic activity, cell part, organelles, and others. In the present study, the downregulation of PRUNE1, NBPF1, PPCaspase-1, UPF2, and MBTPS1 suggested that they inhibited the growth of HepG2 cells. For instance, PRUNE1 is a member of the Asp-His-His phosphoesterase protein superfamily, which is involved in cell motility and is implicated in cancer progression (57). NBPF1 is a tumor suppressor in several cancer types and can act as a tumor suppressor modulating cell apoptosis, possibly through the inhibition of various proteins involved in the cell cycle (58). NBPF1 is also implicated in cancer progression (59). PPCaspase-1 has also been reported to be upregulated in human colon cancer cells. Accordingly, small interfering RNA-mediated PPCaspase-1 knockdown resulted in cell apoptosis in those cells (60). Therefore, precise modulations of the expression level of these critical genes leads to accurate regulation of cellular activity, thereby contributing to the suppressed initiation of cancer progression. Notably, future progress in identifying the basic features of these essential proteins may provide further insights into the diagnosis and prognosis of certain types of human cancer and may also aid the production of novel strategies to develop more effective and efficient therapeutic agents against those types of cancer.

To conclude, 2- and 24-h borax treatment caused significant alterations in the expression levels of various genes. However, based on the length of treatment different sets of genes were altered. Dysregulated genes were identified to be involved in various critical signaling pathways underlying biological processes, including the inflammatory response, stress response, cell apoptosis, signal transduction and cell-to-cell signaling. Some of these changes in those biological processes persisted 24 h after treatment. Thus, it was demonstrated that borax could induce significant alterations in gene expression. However, further studies are required to determine whether these changes are ascribed to genetic alterations in the promoter or regulatory regions of dysregulated genes. Notably, these studies could bring further insights into how borax affects gene expression. The present study provides the fundamental basis for exploring the carcinogenicity of borax treatment in HepG2 to reveal the underlying cellular and molecular mechanisms, the basic

Figure 5. The decreased expression of genes was established following transfection with each shRNA with real-time PCR. *P<0.05, vs. shControl. shRNA, short hairpin RNA.
Figure 6. HepG2 cells were transfected with RNAiMax and counts of live adherent HepG2 in cell culture using a Celigo cytometer at the time points indicated. (A) GFP expression of cells infected with different AdGFP-iRNA. (B) Graphs indicated the number of viable cells. (C) Graphs indicated cell growth according to fold change [fold change = shControl/experimental group (transfected with RNAiMax) ≥1.5, P<0.05]. Ctrl, non-targeting shRNA; PC, positive control (specific-targeting shRNA); AdGFP, adenoviral green fluorescent protein.
biological characteristics and associated pathways, which warrant further investigation.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. 

Authors’ contributions

LW, YW, ZGT and YSZ conceived and designed the experiments. LW, WBZ, QHC, LHY and MXL analyzed and interpreted the experimental data. LW was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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