GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Effect of the ARG1 gene on arsenic resistance of 293T cells

X.L. Mu[1], L. Li[2] and H. Li[3]

[1]Department of Histology and Embryology, Medical College, Shihezi University, Shihezi, Xinjiang, China
[2]Shanghai Research Center for Liver Disease, Eighth PLA Nanjing Military Region, Shanghai, China
[3]Department of Histology and Embryology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Corresponding author: X.L. Mu
E-mail: xlmu2000@yeah.net

Genet. Mol. Res. 12 (4): 6825-6837 (2013)
Received November 14, 2012
Accepted May 13, 2013
Published December 19, 2013
DOI http://dx.doi.org/10.4238/2013.December.19.1

ABSTRACT. To study the relationship between arsenic resistance of 293T cells and overexpression of ARG1, the ARG1 gene in a recombinant plasmid was transfected into 293T cells via liposomes, and then ARG1 overexpression was examined by real-time PCR and immunocytochemistry. The survival rate, arsenic accumulation and arsenic efflux, GSH level, and GST activity of 293T cells overexpressing ARG1 were assayed by MTT, atomic absorption spectrophotometry, and DTNB, and expression of MRP-2 was detected by Western blot analysis. Compared to that in the control cells, the survival rate of ARG1 gene-overexpressing cells was much higher following exposure to lower sodium arsenite (≤8 μM). When cells were exposed to lower sodium arsenite for 24 h, the arsenite content of ARG1 gene-overexpressing cells decreased and arsenic efflux increased. After 48 h, the GSH level, GST activity, and expression of MRP2 increased in a concentration-dependent manner.

We conclude that the ARG1 gene increases arsenic resistance of 293T cells.

Key words: ARG1 gene; Transfection; Arsenic resistance; Glutathione; Glutathione-S-transferase; MRP2

INTRODUCTION

Arsenic is a toxic substance that is widely distributed in the natural environment and has miscellaneous impacts on the organism. The strong toxicity of arsenic poisoning can cause serious damage to cells and organisms; although the pharmacological effects of arsenic have been used to treat many diseases (Jomova et al., 2011). However, prolonged exposure to an arsenic environment can almost spontaneously lead to development of various degrees of resistance to arsenic as an adaptation to the living conditions. Organisms subjected to long-term exposure to arsenic environments at low doses can discharge arsenic from the cell while significantly improving their tolerance to larger doses of arsenic (Rosen, 2002). Studies have shown that resistance to arsenic is mediated by the corresponding gene (Tamas and Wysocki, 2001).

The bacterial genes involved in arsenic resistance are organized in ars operons; most of these operons consist of three genes, arsR, arsB, and arsC. ArsR is an As (III)-responsive transcriptional repressor (Xu et al., 1998). ArsB, or ACR3, is a reverse transporter (antiporter) of As (OH) 3/H- and can pump As (III) out of the cell so that the cells expressing this gene have anti-arsenic properties (Stamatelos et al., 2011). ArsC is an arsenate reductase that catalyzed the reduction of pentavalent arsenic [As (V)] to trivalent arsenic [As (III)], thereby conferring the cells with resistance to pentavalent arsenic (Mukhopadhyay and Rosen, 2002). Part of the arsC gene also contains arsD [a metal chaperone-transporting As (III) to ArsA] and arsA [the catalytic subunit of arsAB, the As (III) efflux pump] (Lin et al., 2006; Achour-Rokbani et al., 2010). For example, the gene responsible for arsenic resistance in *Escherichia coli* plasmid R773 is composed of the arsRDABC operon; ArsA and ArsB form complexes, utilize the energy generated by ATP hydrolysis, and discharge As (III) or Sb (III) (Ajees et al., 2011). Resistance to As (V) and As (III) is higher in cells expressing five genes (arsRDABC) than in cells expressing only arsRBC (Ajees et al., 2011). Another report showed that the linear plasmid pHZ227 of *Streptomyces* sp (FR-008), which confers arsenic resistance, carries an anti-arsenic gene cluster arsRBOCT containing two new genes, arsO and arsT, which are co-activated and co-transcribed with arsR1-arsB-arsC. These two new genes encode a new putative flavin-binding monooxygenase (arsO) and a putative thioredoxin reductase (arsT) (Wang et al., 2006). The *Glutamic acid Corynebacterium* (ATCC 13032) strain was recently shown to possess two members of a new class of arsenate reductases (Cg_ArsC1 and Cg_ArsC2) (Ordóñez et al., 2009) and a transcriptional repressor (Cg_ArsR1) with a metalloid binding site unrelated to other previously characterized members of the ArsR/SmtB metalloregulatory proteins (Ordóñez et al., 2008). An original set of arsenic resistance genes isolated from *Microbacterium* sp (strain A33) was previously shown to tolerate high concentrations of arsenite and arsenate. Distinctive attributes of the *ars* cluster include the placement of two genes encoding a thioredoxin system within the cluster, one gene encoding a putative thioredoxin-coupled arsenate reductase lacking two catalytic cysteines, and a fusion gene whose product is a multidomain protein consisting of a transcription factor and an arsenate reductase (Achour-Rokbani et al.,

2010). The *Bacillus subtilis skin* element confers resistance to arsenate and arsenite. The *ars* operon in the *skin* element contains four genes in the order *arsR*, *ORF2*, *arsB*, and *arsC*. Three of these genes are homologous to the *arsR*, *arsB*, and *arsC* genes from the *staphylococcal* plasmid pI258, while no homologs of *ORF2* have been found. Inactivation of *arsR*, *arsB*, or *arsC* results in either constitutive expression of *ars*, an arsenite- and arsenate-sensitive phenotype, or an arsenate-sensitive phenotype, respectively. These results suggest that ArsR, ArsB, and ArsC function as a negative regulator, a membrane-associated protein need for extrusion of arsenite, and arsenate reductase, respectively (Sato and Kobayashi, 1998). In *Halobacterium* sp (strain NRC-1), the large extrachromosomal replicon pNRC100 contains an arsADRC-R2M arsenic resistance gene cluster, which is associated with the trivalent arsenic-methyltransferase (Wang et al., 2004). The circular plasmid pWCFS103 of *Lactobacillus plantarum* carries an arsenic resistance gene cluster, arsRDDB, which has a unique composition because it lacks the pentavalent arsenic reductase gene arsC and contains two copies of the arsD-regulated gene (Kleerebezem et al., 2003).

In the eukaryotic model organisms *Saccharomyces cerevisiae* and *Saccharomyces douglasii*, anti-arsenic gene clusters are composed of ACR1, ACR2, and ACR3; their functions are respectively similar with those of bacterial arsR, arsC, and arsB; however, the product sequences of the three genes are completely different from those of the bacterial counterparts (Wang et al., 2006). Takahashi et al. (2010) reported that they transfected the open reading frame plasmid of budding yeast into *S. cerevisiae* and screened four genes associated with arsenic tolerance, FAP7, MIG3, TMA19, and YLR392c. Among them, FAP7 is an NTPase of ribosome biosynthesis; MIG3 is a transcriptional repressor in glucose metabolism; Tma19 is a ribosome-binding factor in translation; and Y1r392c is a protein with unknown function.

The present findings show that intake and excretion of arsenic primarily depend on the transporter protein. For example, liver cells take in inorganic trivalent arsenic through the water channel protein aquaporin isozyme 9 (AQP9) while discharging single-methylated trivalent organic arsenic monomethylated arsenical (MMA) (Hachez and Chaumont, 2010; Rosen and Tamas, 2010; Stamatelos et al., 2011). Another kind of transporter protein is the glucose transporter, in particular, GLUT2 that is highly expressed in the liver (Stamatelos et al., 2011). Glutathione-conjugated arsenicals are exported to the extracellular space via multidrug resistant proteins (MRPs) and multidrug resistant P-glycoproteins (PGPs), which are ATP-binding cassette transporters that export solutes against their concentration gradient (Stamatelos et al., 2011). In liver cells, inorganic arsenic is methylated into MMA and dimethyl arsenic by the arsenic methyltransferase AS3MT through two pathways. GSH exerts stimulating effects in both these methylation pathways, whereas arsenic activates and recruits the redox-sensitive transcription factor nuclear factor-E2-related factor 2 (Nrf-2) into the nucleus and binds to the antioxidant response element, which stimulates liver cells to exert antioxidation effects (Stamatelos et al., 2011). However, a definite anti-arsenic key gene has not yet been found. The ARG1 (arsenite-related gene 1) gene is a novel cloned gene isolated from human fetal brain. Its total length is 2255 bp, and its open reading frame (ORF) is 1532 bp. Previous studies have shown that the ARG1 gene is expressed the least or almost not expressed in L-02 cell lines without induction by arsenic; however, 2 weeks after induction with arsenic, ARG1 expression was significantly increased in an arsenic dose-dependent manner. So ARG1 was considered a novel human gene with arsenic antagonistic properties (Rosen and Tamas, 2010).

In this study, based on the results from previous researches, the eukaryotic expression vector containing the ORF of the ARG1 gene was transfected into 293T cells, and the changes in the viability of the transfected 293T cells, accumulation volume of intracellular arsenic, excretion volume of arsenic, and levels of the enzymes and proteins related to arsenic metabolism were observed to determine the relationship between arsenic resistance and genes in cells.

MATERIAL AND METHODS

Reagents

α-DMEM medium (high glucose) and fetal bovine serum (FBS) were purchased from Gibco; Lipofectamine 2000 reagents were purchased from Invitrogen; and the DNA plasmid extraction kit was purchased from Takara (TaKaRa, Dalian, CHINA). Tetrazolium salt (MTT) was purchased from Beijing Huamei Biotechnology in China. Dimethyl sulfoxide (DMSO), sodium arsenite ($NaAsO_2$), and trypsin were purchased from Sigma (Sigma, St. Louis, MO, USA). The GSH and GST assay kits were purchased from Nanjing Jiancheng Institute of Biotechnology in China; mouse anti-human MRP2 antibody (Santa Clara, CA, USA). Horseradish peroxidase-conjugated goat anti-mouse IgG and β-actin antibody were purchased from Beijing Zhongshan Golden Bridge in China.

Plasmids and cells

Human embryonic kidney cells (293T cells) were kept in our laboratory, and PcDNA3.1-IE-ARG1 plasmid was built in our laboratory; the pcDNA3.1-IRES-EGFP plasmid was purchased from Pan Keno in Beijing Science and Technology Co., Ltd.

Plasmid extraction

PcDNA3.1-IE-ARG1 plasmids and pcDNA3.1-IRES-EGFP plasmids were extracted and purified in small amounts, and the extraction was performed according to the instructions in the kit used.

293T cells

293T cells were cultured in anti-double H-DMEM medium (Gibco, USA) containing 10% FBS at 37°C and 5% CO_2.

Construction of recombinant DNA

The PCR primer with *Bam*HI and *Eco*RI restriction sites is 5'-AGGATCCCCAATGG AGGCAGATATAATCAC-3' (the basic radicals underlined are the *Bam*HI restriction site) and the ORF of amplified ARG is 5'-AAAGAAAAGTCTTTACTGTAGGG-3'. Amplified fragments were cut with *Bam*HI and *Eco*RI and inserted into the pcDNA3.1-IRES-EGFP vector (Beijing Pan Keno Technology Co., Ltd.).

Transfection of 293T cells

The liposome-mediated transfection method was adopted. Three experimental groups were created: target gene group, empty vector group, and non-transfected group. For each group 5 x 10^5 cells/well were seeded on a 6-well plate and cultured for 24 h. After incubation, when the cell density reached 70-80%, the cells were washed once with PBS, and then DMEM without serum or antibiotics was added and the cells were cultured for further 24 h. Two micrograms of recombinant plasmid pcDNA3.1-IE-ARG1 and empty vector pcDNA3.1 was added, along with 50 µL serum and antibiotic-free H-DMEM (liquid A) and 8 µL Lipofectamine 2000 with 250 µL H-DMEM (liquid B). After mixing for 5 min, the pre-cultured 293T cells on the 6-well plates were washed once with PBS, the transfection liquid mixture was added to the plates, and the plates were incubated at 37°C and 5% CO_2 for 6 h; then, the medium was replaced with H-DMEM containing 10% FBS and antibiotics and culturing was continued. The condition of cell transfection was observed under a laser confocal microscope, and gene expression was detected by real-time PCR. The cells in each group were treated with 0, 4, and 8 µM medium containing NaAsO2 and cultured for 48 h to detect expression of the ARG1 gene.

Determination of the survival rate of 293T cells by the 48-h arsenic toxicity assay

293T cells with good growth, 293T cells transfected with the recombinant plasmid, and the 293T cells transfected with the empty vector were seeded on 96-well plates at 5000 cells/well, and cultured at 37°C and 5% CO_2; 24 h after adherence of cells, the culture media were replaced with H-DMEM containing $NaAsO_2$ at concentrations of 0 (control), 1, 2, 4, 8, 16, and 32 µM. Five wells were designated for each concentration, and the cells were cultured for further 48 h, after which the medium was discarded. MTT solution (5 mg/mL) was added to each well, the cells were incubated for 4 h, and observed under an inverted microscope. The used medium was discarded, DMSO was added at 100 µL/well, gently oscillated to dissolve the crystals, and the plates were kept at room temperature for 10 min. The absorbance value (OD) at 490 nm was determined using an automatic microplate reader, and the survival rates of the cells in the experimental and control groups at various concentrations of $NaAsO_2$ were calculated according to the OD value of cells: survival rate = (OD values of arsenic wells - OD value of zero well) / (OD values of control wells - OD value on zero well) x 100%; inhibition rate = 1 - survival rate.

Determination of accumulation and discharge of arsenic in 293T cells

The cells from the above three groups were seeded on nine 100-mm culture plates at 2 x 10^7 cells/mL, 3 dishes per group. Four milliliters of arsenic-free H-DMEM (containing 10% FBS) was added to each dish and the cells were cultured for 24 h; subsequently, the medium was replaced with H-DMEM (containing 10% FBS) and $NaAsO_2$ at a concentration of 1, 4, and 8 µM. After 24 h of culture, the medium was collected in a centrifuge tube and the cells were washed three times with PBS. Then, arsenic-free H-DMEM (containing 10% FBS) was added and the cells were cultured for 24 and 48 h, then the culture medium was collected in a dish. The arsenic content in the culture medium was determined by atomic fluorescence spectrometry for indirect measurement of accumulation and excretion of arsenic from 293T cells.

Detection of intracellular GSH and GST in 293T cells

GSH and GST were determined by using the disulfide double-nitrobenzoic acid method. The cells from the three groups were seeded on nine 100-mm culture plates at 2 x 10^7 cells/mL. Four milliliters of arsenic-free H-DMEM (containing 10% FBS) was added to each dish and cultured for 24 h. After incubation, the medium was replaced with H-DMEM (containing 10% FBS) and $NaAsO_2$ at a concentration of 0, 4, and 8 μM. After 48 h of culture, the medium was discarded and the cells were washed twice with PBS. After homogenization on ice bath and centrifugation at 15,000 rpm for 10 min at 4°C, the levels of intracellular-reduced glutathione (glutathiome) and glutathione peptide-S-transferase enzymes (glutathione-S-transferase) were assayed according to the instructions on the kits used.

Western blot analysis for detection of MRP2 protein expression in 293T cells

The cell lysates of the three groups were collected to determine the protein concentration; 35 μL total protein was taken, and subjected to electrophoresis on a 6% separating gel. After electrophoresis, the protein was transferred to nitrocellulose membrane, and the membrane was blocked with the blocking solution (5% skim milk-TBST solution) for 1-1.5 h. Then, the membrane was incubated first with primary antibody (mouse anti-human MRP2 antibody, 1:500) and then with the secondary antibody (horseradish peroxidase-conjugated goat anti-mouse IgG, 1:2000) for 1-1.5 h each. The DAB color system was used for color development, and the hybridization bands were observed and analyzed with QUANTITY ONE and GEL-DOC2000 (BIORAD).

Statistical analysis

Data are reported as means ± standard deviation. The SPSS13.0 software was used for statistical analysis; the data from different groups were compared using single factor analysis of variance (one-way ANOVA, LSD). $P < 0.05$ was considered to be statistically significant.

RESULTS

Expression of PcDNA3.1-IE-ARG1 in 293T cells

Because the recombinant plasmid PcDNA3.1-IE-ARG1 (Figure 1) was labeled with enhanced green fluorescent protein (EGFP), EGFP expression, which can be observed by laser confocal microscopy, can indirectly reflect the transfection rate of the ARG1 gene; 24 h after transfection, green fluorescence could be seen in the recombinant plasmid EGFP-specific cells, and GFP was distributed in the cell membrane. The transfection rate was 70% or higher; the GFP in the cells from the empty vector group was distributed throughout the cell (Figure 2).

To confirm that the gene had been transfected into cells, we used real-time PCR to detect ARG1 mRNA expression in recombinant cells. The results showed that the standard curve of ARG1 in real-time PCR had good linear correlation, and the amplification curve showed an index number amplification with an "S" shape; the conditions corresponded with the relative quantification standards (two-way standard curve quantitative method). After treatment with $NaAsO_2$ at 0, 4, and 8 μM for 48 h, relative expression of the ARG1 gene in recombinant cells

was 2.83 x 10^{-3} ± 0.2 x 10^{-3}, 3.07 x 10^{-3} ± 0.2 x 10^{-3}, and 3.58 x 10^{-3} ± 0.17 x 10^{-3}, respectively (Figure 3). With the increasing concentration of arsenic, ARG1 mRNA expression also increased in a dose-dependent way. ARGI gene expression was not detected in the cells from the empty vector or the non-transfection groups. This result also demonstrated that the target gene had been successfully transfected.

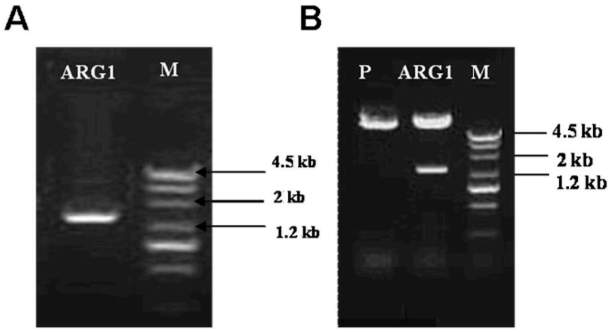

Figure 1. A. Electropherogram of colony PCR products of plasmid transformation bacteria. **B.** Electropherogram of enzyme digestion of recombinant plasmid. *Lane M* = molecular marker; *lane P* = empty vector pcDNA3.1.

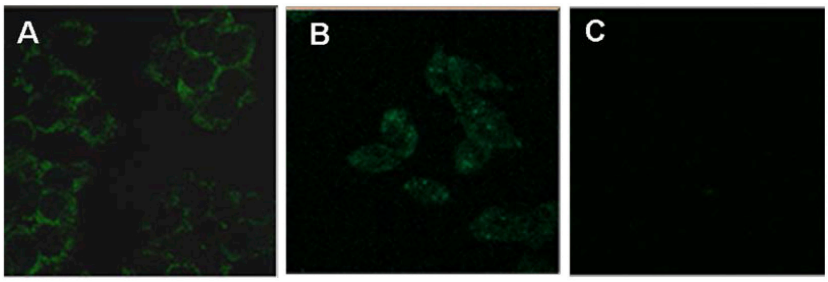

Figure 2. Expression of ARG1 in 293T cells in confocal microscopy. **A.** ARG1 gene group; **B.** empty vehicle group; **C.** control group.

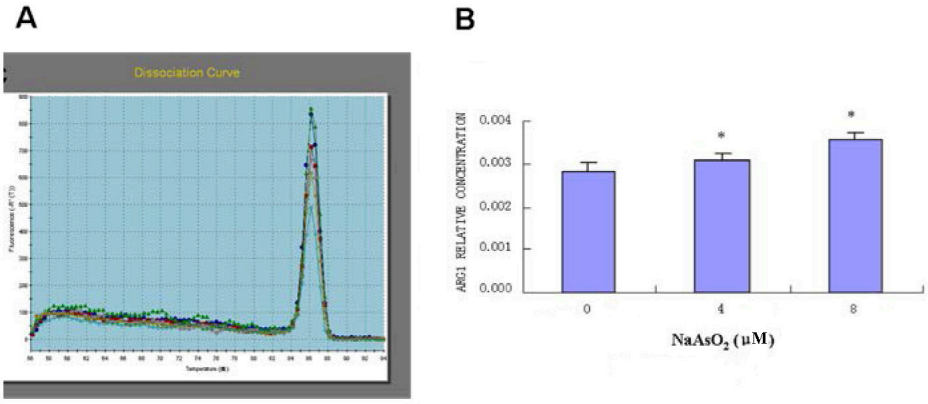

Figure 3. Relative expression of the ARG1 gene in 293T cells after 48 h of treatment with $NaAsO_2$ at different concentrations. N = 3,*P < 0.05.

Forty-eight-hour acute arsenic poisoning test

In order to understand whether the ARG1 gene affects the tolerance of 293T cells to high arsenic, we used the MTT assay and determined the survival rates of cells exposed to 48-h acute arsenic poisoning. The cells in each group were cultured in medium containing $NaAsO_2$ at various concentrations for 48 h; the results from the MTT assay (Table 1) showed that with increasing concentrations of $NaAsO_2$, the survival rates of the cells reduced gradually, showing that cell growth was inhibited in a dose-dependent manner (Figure 4). When the concentration of $NaAsO_2$ was ≤8 μM, the survival rate of the cells from the target gene group was significantly higher than that of the cells from the non-transfected and empty vector-transfected groups; the difference was statistically significant ($P < 0.05$).

Table 1. Results of the effect of ARG1 on the survival rates in the cells of each group.

Groups	1 μM	2 μM	4 μM	8 μM	16 μM	32 μM
Objective gene	104.22 ± 0.63	102.94 ± 0.40	98.09 ± 0.97	77.01 ± 0.23	35.20 ± 1.82	23.56 ± 1.20
Non-transfection	87.16 ± 1.80	83.39 ± 0.15	78.13 ± 5.25	68.01 ± 2.19	36.01 ± 0.65	22.32 ± 1.14
Empty vehicle	84.17 ± 2.15	80.39 ± 2.05	71.13 ± 1.31	67.16 ± 2.20	34.41 ± 1.45	21.32 ± 1.52

Data are reported as means ± SD.

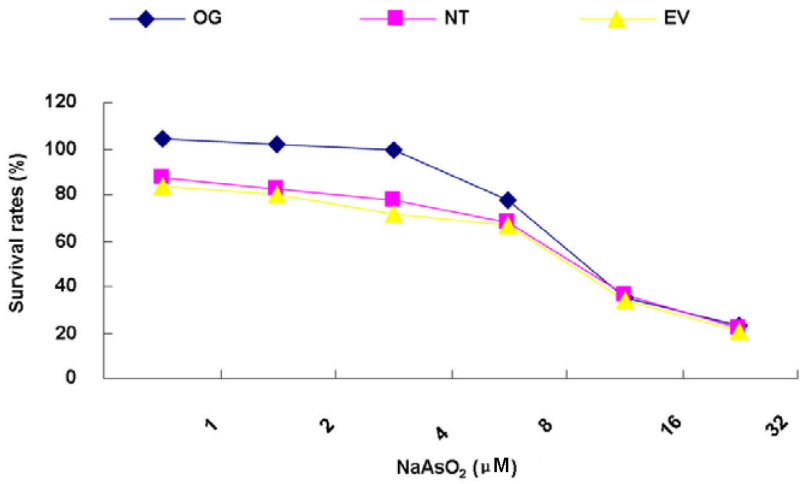

Figure 4. Comparison of survival rates of the cells in each group. OG = objective gene group; NT = non-transfection group; EV = empty vehicle group.

Determination of intracellular arsenic accumulation and volume of arsenic discharge

Both acquisition of cellular resistance to arsenic and enhancement of the arsenic discharge ability were related to reduction of intracellular accumulation of arsenic. Therefore, to observe whether the ARG1 gene could affect the arsenic content and the arsenic discharge ability of cells, we treated cells from the target gene group, non-transfection group, and empty vector group with different concentrations of arsenic. The intracellular arsenic concentrations for all

the three groups were determined. The results showed that the arsenic accumulation in the transfected cells increased with the increase in arsenic doses ($P < 0.05$), but arsenic accumulation in the target gene group was significantly lower than that in the other two groups ($P < 0.05$) (Figure 5). We then detected the amount of arsenic excreted 24 and 48 h after treatment with arsenic. The results showed that the arsenic discharge capacity of cells from each group decreased gradually with increase in arsenic doses. Further, the arsenic discharge capacity of the cells from the target gene group was significantly higher than that of the other two groups; this difference was statistically significant ($P < 0.05$), although there was no significant difference between the amount of arsenic discharged from the cells in the empty vector-transfected group at 24 and 48 h (Figure 5).

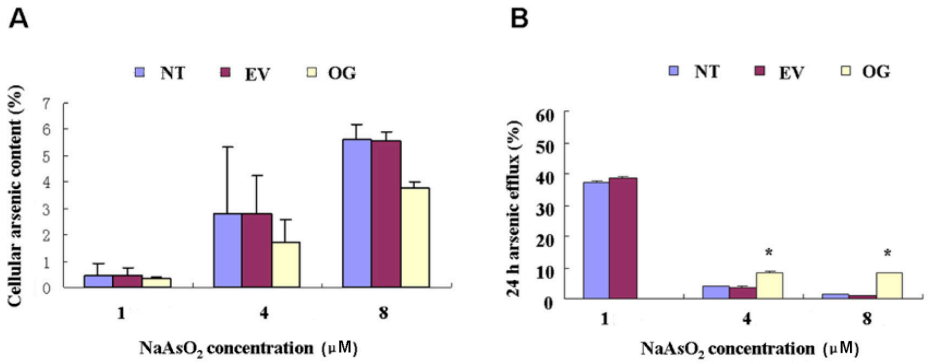

Figure 5. Cellular arsenic content and 24 h arsenic efflux of $NaAsO_2$ at different concentrations. NT = non-transfection group; EV = empty vehicle group; OG = objective gene group.

Assay of GSH content and GST activity

To investigate the mechanism underlying arsenic resistance conferred by the target gene, we determined the intracellular GSH content and GST activity of cells from the target gene group, non-transfected group, and empty vector group. The results showed that when the concentration of $NaAsO_2$ was 0, there was no significant difference in intracellular GSH content and GST activity among the three groups. However, after treatment with arsenic, the intracellular GSH content and GST activity of the cells from the target gene group were significantly higher than those of the cells from the non-transfected and empty vector groups, and this difference was statistically significant ($P < 0.05$) (Table 2).

Table 2. Comparison of the effect of ARG1 on GSH and GST content in the cells of each group.

Groups	Arsenium (μM)	GSH content (g/g protein)	GST content (U/mg protein)
NT	0	5.51 ± 0.09	55.91 ± 1.0
	4	7.48 ± O.14	64.05 ± 2.74
	8	8.63 ± 0.01	74.22 ± 1.21
OG	0	5.46 ± 0.03	54.83 ± 0.75
	4	9.79 ± 0.23*	102.34 ± 5.74*
	8	10.56 ± 0.14*	165.11 ± 7.83*
EV	0	5.18 ± 0.34	51.27 ± 4.68
	4	7.06 ± 0.70	63.53 ± 3.62
	8	8.61 ± 0.97	73.7 ± 2.1

Data are reported as means ± SD. *$P < 0.05$, compared with non-transfection group and empty vehicle group. NT = non-transfection group; OG = objective gene group; EV = empty vehicle group.

Expression of the MRP2 protein in 293T cells before and after transfection

In order to determine the gene expression levels of the MRP2 protein, we determined MRP2 protein expression using Western blot analysis. The results showed that MRP2 protein expression was significantly higher in the cells transfected with plasmid at each concentration (0, 4, 8 μM) than in the control cells ($P < 0.05$). Moreover, MRP2 expression increased with the increase in arsenic concentrations (Table 3 and Figure 6).

Table 3. Quantitive analysis results of the effect of ARG1 on MRP2 expression in the cells of each group.

Groups	Arsenium (μM)	Relative expression of MRP2
NT	0	0.46 ± 0.01
	4	0.51 ± O.01
	8	0.72 ± 0.02
OG	0	0.45 ± 0.017
	4	0.49 ± O.021
	8	0.70 ± 0.03
EV	0	0.65 ± 0.023*
	4	0.91 ± 0.026*
	8	0.98 ± 0.017*

Data are reported as means ± SD. *$P < 0.05$, compared with non-transfection group and empty vehicle group. NT = non-transfection group; OG = objective gene group; EV = empty vehicle group.

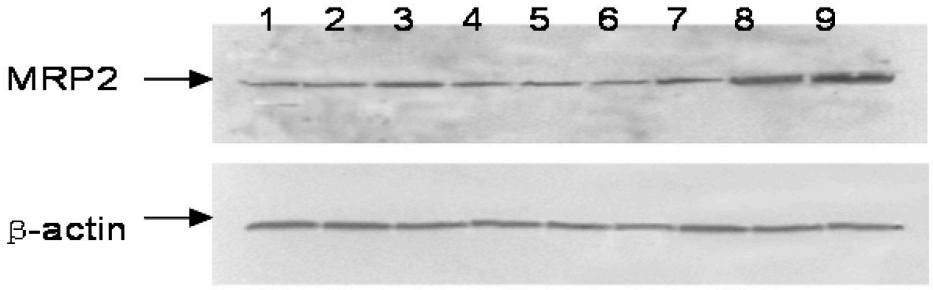

Figure 6. Effect of the ARG1 gene on protein expressions of MRP2 in 293T cells. *Lanes 1-3* = non-transfection group; *lanes 4-6* = empty vehicle group; *lanes 7-9* = objective gene group.

DISCUSSION

The present findings showed that intake and excretion of arsenic are primarily mediated by transporter proteins. For example, liver cells take in inorganic trivalent arsenic through the water channel protein AQP9 while discharging single-methylated trivalent organic arsenic MMA (Takahashi et al., 2010; Hachez and Chaumont, 2010; Stamatelos et al., 2011).

In this study, the ARG1 gene was transfected into 293T cells, and after treatment of the recombinant cells with different concentrations of NaAsO2 for 48 h, the expression of the ARG1 gene increased with increasing doses of arsenic. Our results confirmed the previous findings (Yong-Qing et al., 2000); 48-h acute toxicity test results showed that at low doses of arsenic, the ARG1 gene functions to increase the arsenic tolerance of cells; however, these findings are only preliminary evidence that the gene confers resistance to arsenic.

To further confirm arsenic resistance of the ARG1 gene, we determined the intracellular arsenic content, arsenic discharge capacity, GSH content, GST activity, and MRP2 protein expression levels in 239T cells before and after transfecting the ARG1 gene and incubation with arsenic at 1, 4, and 8 μM for 24 and 48 h. The results showed that arsenic accumulation in the cells from the target gene group reduced significantly and the arsenic discharge capacity of the cells increase significantly after 24 and 48 h, which corresponded with the results of Brambila et al. (2002). Brambila et al. (2002) considered that the antiarsenic capacity of cells was due mainly to increase in arsenic excretion, which in turn results in decrease of intracellular arsenic accumulation. However, intracellular arsenic levels in the cells before and after transfection increased with the increase in arsenic concentrations, whereas the arsenic discharge capacity decreased gradually. In addition, this study also found that the intracellular GSH content and GST activity of the target gene group were significantly higher than those in the cells from the other two groups, and the difference was statistically significant ($P < 0.05$). Brambila et al. (2002) used 30 μM arsenic for acute treatment of the arsenic cell line CAsE-PE; in that study too, the intracellular GSH concentration and GST activity increased. The studies of Romach et al. (2000) suggested that the arsenic discharge capacity of mammalian cells was related to elevated GSH and GST levels. GSH can enhance arsenic pump activity, increase intracellular arsenic excretion, and reduce intracellular arsenic toxicity. GST, especially GST-π, may promote formation of arsenic complexes with GSH, thereby promoting arsenic discharge from cells. GSH is an important antioxidant, and in arsenic metabolism, trivalent arsenic in combination with GSH can form arsenic-sulfur compounds to accelerate arsenic excretion. In addition, GSH is also involved in methylation reduction in inorganic arsenic metabolism (Romach et al., 2000; Drobna et al., 2010). GST is one of the metabolic enzymes involved in metabolic detoxification of exogenous chemical materials. On the one hand, it can catalyze combination of harmful polar compounds with glutathione in the body, and on the other hand, it is a binding protein, binding to potentially toxic chemical materials, carcinogens, and lipophilic compounds with high affinity in the body, thereby helping in their excretion from the body. All of these clarify that GST participates in antioxidation and cellular synthesis, storage, and transport of many substances. It is a major detoxification system in many organisms (Romach et al., 2000). Our experimental results demonstrate that the ARG1 gene can significantly enhance the resistance of cells to arsenic at low concentrations for short durations and promote metabolism and excretion of arsenic.

The expression level of MRP2 was significantly higher in cells transfected with the ARG1 gene than in cells from the other two groups; the difference was statistically significant ($P < 0.05$). Previous reports showed that arsenic in combination with glutathione can be excreted by MRPs and PGPs (Li et al., 2007; Drobna et al., 2010; Carew and Leslie, 2010). Li et al. (2007) have shown that when cells subjected to long-term exposure to a low-dose arsenic environment are subjected to acute arsenic exposure their sensitivity to arsenic reduces significantly. In this process, high expression of MRP2 can be detected, and arsenic can be pumped out of the cell, thereby enhancing arsenic tolerance of the cells. The results suggested that the ARG1 gene can facilitate discharge of arsenic by enhancing multi-drug resistance-associated protein and the functions of the glutathione transporter system, thereby improving the arsenic tolerance of cells. Certain synergies may exist between MRP2 and glutathione transporter system. MRP is a recently discovered transmembrane glycoprotein, belonging to the multigene family of ATP-binding membrane proteins. Studies have shown that when As (III) exists in

the cells, MRP1 catalyzes As (GS) 3 to enhance arsenic excretion from cells. GS-As (III) compounds are excreted into the bile in the liver. In addition, after the effect of MRP is blocked with MRP blockers, the antiarsenic capacity of the arsenic-resistant cell line CAsE-PE disappears, which indicates that MRP can promote discharge of arsenic in mammalian cells, which is key to development of arsenic tolerance in cells (Brambila et al., 2002). Other data showed that under physiological conditions, there was a glutathione output system dependent on MRP2; endogenous toxic substances were pumped out of the cell by the MRP2 and GSH transporter mechanism, and intracellular GSH levels can adjust the active transport of MRP2 to the substrate (Zaman et al., 1995). Our study preliminarily demonstrated that high expression of the ARG1 gene can enhance the tolerance of 293T cells to arsenic and that ARG1 is an antiarsenic gene. It exerts some effects in the process of arsenic excretion, and we speculate that the ORF encoding the protein may play an important role in the transport and excretion of arsenic; however, the specific mechanism underlying these effects remains to be elucidated.

REFERENCES

Achour-Rokbani A, Cordi A, Poupin P, Bauda P, et al. (2010). Characterization of the ars gene cluster from extremely arsenic-resistant *Microbacterium* sp. strain A33. *Appl. Environ. Microbiol.* 76: 948-955.

Ajees AA, Yang J and Rosen BP (2011). The ArsD As(III) metallochaperone. *Biometals* 24: 391-399.

Brambila EM, Achanzar WE, Qu W, Webber MM, et al. (2002). Chronic arsenic-exposed human prostate epithelial cells exhibit stable arsenic tolerance: mechanistic implications of altered cellular glutathione and glutathione S-transferase. *Toxicol. Appl. Pharmacol.* 183: 99-107.

Carew MW and Leslie EM (2010). Selenium-dependent and -independent transport of arsenic by the human multidrug resistance protein 2 (MRP2/ABCC2): implications for the mutual detoxification of arsenic and selenium. *Carcinogenesis* 31: 1450-1455.

Drobna Z, Walton FS, Paul DS, Xing W, et al. (2010). Metabolism of arsenic in human liver: the role of membrane transporters. *Arch. Toxicol.* 84: 3-16.

Hachez C and Chaumont F (2010). Aquaporins: a family of highly regulated multifunctional channels. *Adv. Exp. Med. Biol.* 679: 1-17.

Jomova K, Jenisova Z, Feszterova M, Baros S, et al. (2011). Arsenic: toxicity, oxidative stress and human disease. *J. Appl. Toxicol.* 31: 95-107.

Kleerebezem M, Boekhorst J, van Kranenburg R, Molenaar D, et al. (2003). Complete genome sequence of *Lactobacillus plantarum* WCFS1. *Proc. Natl. Acad. Sci. U. S. A.* 100: 1990-1995.

Li GX, Pei QL, Gao Y, Liu KM, et al. (2007). Protective effects of hepatocellular canalicular conjugate export pump (Mrp2) on sodium arsenite-induced hepatic dysfunction in rats. *Exp. Toxicol. Pathol.* 58: 447-453.

Lin YF, Walmsley AR and Rosen BP (2006). An arsenic metallochaperone for an arsenic detoxification pump. *Proc. Natl. Acad. Sci. U. S. A.* 103: 15617-15622.

Mukhopadhyay R and Rosen BP (2002). Arsenate reductases in prokaryotes and eukaryotes. *Environ. Health Perspect.* 110 (Suppl 5): 745-748.

Ordóñez E, Thiyagarajan S, Cook JD, Stemmler TL, et al. (2008). Evolution of metal(loid) binding sites in transcriptional regulators. *J. Biol. Chem.* 283: 25706-25714.

Ordóñez E, Van Belle K, Roos G, De Galan S, et al. (2009). Arsenate reductase, mycothiol, and mycoredoxin concert thiol/disulfide exchange. *J. Biol. Chem.* 284: 15107-15116.

Romach EH, Zhao CQ, Del Razo LM, Cebrian ME, et al. (2000). Studies on the mechanisms of arsenic-induced self tolerance developed in liver epithelial cells through continuous low-level arsenite exposure. *Toxicol. Sci.* 54: 500-508.

Rosen BP (2002). Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 133: 689-693.

Rosen BP and Tamas MJ (2010). Arsenic transport in prokaryotes and eukaryotic microbes. *Adv. Exp. Med. Biol.* 679: 47-55.

Sato T and Kobayashi Y (1998). The ars operon in the skin element of *Bacillus subtilis* confers resistance to arsenate and arsenite. *J. Bacteriol.* 180: 1655-1661.

Stamatelos SK, Brinkerhoff CJ, Isukapalli SS and Georgopoulos PG (2011). Mathematical model of uptake and metabolism of arsenic(III) in human hepatocytes - Incorporation of cellular antioxidant response and threshold-dependent behavior. *BMC Syst. Biol.* 5: 16.

Takahashi T, Yano T, Zhu J, Hwang GW, et al. (2010). Overexpression of FAP7, MIG3, TMA19, or YLR392c confers resistance to arsenite on *Saccharomyces cerevisiae*. *J. Toxicol. Sci.* 35: 945-946.

Tamas MJ and Wysocki R (2001). Mechanisms involved in metalloid transport and tolerance acquisition. *Curr. Genet.* 40: 2-12.

Wang G, Kennedy SP, Fasiludeen S, Rensing C, et al. (2004). Arsenic resistance in *Halobacterium* sp. strain NRC-1 examined by using an improved gene knockout system. *J. Bacteriol.* 186: 3187-3194.

Wang L, Chen S, Xiao X, Huang X, et al. (2006). arsRBOCT arsenic resistance system encoded by linear plasmid pHZ227 in *Streptomyces* sp. strain FR-008. *Appl. Environ. Microbiol.* 72: 3738-3742.

Xu C, Zhou T, Kuroda M and Rosen BP (1998). Metalloid resistance mechanisms in prokaryotes. *J. Biochem.* 123: 16-23.

Yong-Qing G, Lei Y, Hong-Ling Y, Chao-Qun W, et al. (2000). Cloning and preliminary functional analysis of a novel human full-length cDNA related to gene arsenite metabolism. *J. Fundan Univ.* (*Natural Science*) 39: 603-608.

Zaman GJR, Lankelma J, van Tellingen O, Beijnen J, et al. (1995). Role of glutathione in the export of compounds from cells by the multidrug-resistance-associated protein. *Proc. Natl. Acad. Sci. U. S. A.* 92: 7690-7694.