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Toxicomics Report

Knockdown of Acyl-CoA thioesterase 9 gene expression by siRNA confers resistance to arsenite in HEK293 cells

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ABSTRACT — Arsenic is a hazardous element that exists widely in the environment. To elucidate the molecular mechanism involved in the onset of arsenic toxicity, we comprehensively searched for genes affecting the sensitivity of HEK293 cells to arsenite by using a siRNA library targeting approximately 17,000 human genes. As a result, we identified ACOT9, one of the acyl-coenzyme A thioesterases, as the gene conferring arsenite resistance to cells by knockdown.

Key words: Arsenite, ACOT9, Acyl-Coenzyme A thioesterase

INTRODUCTION

Arsenic exists widely in the environment. Health hazards caused by arsenic pollution of ground water have been issues of global concern, especially in Southeast Asia (Ng and Moore, 2005; Chen *et al*., 2007). The use of an arsenic compound as a therapeutic drug for acute promyelocytic leukemia has caused serious adverse reactions, preventing the effective use of this drug (Hu *et al*., 2005). A number of molecular mechanisms are reported to be involved in the onset of arsenic toxicity, including inhibition of enzyme activity following reaction with enzyme thiol groups (Petrick *et al*., 2001), enhanced generation of reactive oxygen species (ROS) (Kumagai and Pi, 2004; Ellinsworth, 2015), and the promotion of apoptosis following the activation of signaling systems (Shim *et al*., 2002; Kumagai and Sumi, 2007). However, the exact toxicity mechanism remains largely unknown. Thus, in this study we comprehensively searched for genes affecting the sensitivity of cells to arsenite (a molecule with particularly strong toxicity among arsenic compounds) by using a siRNA library targeting approximately 17,000 human genes.

MATERIALS AND METHODS

Cell culture

Human embryonic kidney 293 (HEK293) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/L L-glutamine, 100 IU/mL penicillin, and 100 mg/mL streptomycin in humidified air containing 5%CO_2 and maintained at 37°C.

Screen for arsenite-resistant transfectants with siRNA

Double-stranded small inhibitory (si) RNAs were purchased from Qiagen (Germantown, MD, USA). Transfection of HEK293 cells with siRNAs was carried out as described elsewhere, using the HiperFect transfection reagent (Qiagen) according to the manufacturer's protocol (Hwang *et al.*, 2010, 2011). Briefly, 2.5 μL/well of siRNA mixture (final: 10 nM) containing two kinds of double-stranded siRNA was spotted into the wells of 96-well plates. A total of 0.75 μL of HiperFect transfection reagent was diluted in 24.25 μL of Opti-MEM (Invitorogen, Carlsbad, CA, USA), and the diluted reagent was added to the prespotted siRNA. After a 10 min incu-

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bation at room temperature, HEK293 cells (5×10^3 cells) were plated and cultured in 100 µL aliquots of DMEM. Forty-eight hr after siRNA transfection, the HEK293 cells were treated with 5 µM sodium arsenite for 48 hr. Cell viability was measured via Alamar Blue assay (Biosource, Camarillo, CA, USA) (Takahashi *et al.*, 2006).

Confirmation of knockdown efficiency using quantitative real-time PCR

Double-stranded siRNA targeting the ACOT9 gene (siRNA No. 1, UAGGAGAAUGAAGGACAGUUA; siRNA No. 2, AAGGCAAUGGAAGAAAGGAAA) and AllStars® negative control siRNA were purchased from Qiagen. Forty-eight hr after transfection with siRNAs, cells were gently rinsed with phosphate-buffered saline. Isolation of total RNA from HEK293 cells was carried out using an Isogen kit (Nippon Gene, Tokyo, Japan) according to the manufacturer's protocol. The first strand cDNA was synthesized from 500 ng of total RNA using a PrimeScript™ RT reagent kit (Takara, Shiga, Japan). We performed quantitative real-time PCR (qPCR) using SYBR Premix EX Taq (Takara) and a Thermal Cycler Dice® (Takara) (Kim *et al.*, 2012). The qPCR primers used were as follows: GAPDH, 5'-GGGGAAGCTTGTCAATGG-3' (sense) and 5'-GGCAGTGATGGCATGGACTC-3' (antisense); ACOT9, 5'- CAAAGGGCAGCTTACTCCTG -3' (sense) and 5'- GGCAGTCCATCCTGTGATTT-3' (antisense). Levels of ACOT9 mRNA were normalized to those of GAPDH.

RESULTS AND DISCUSSION

We introduced individual siRNAs targeting approximately 17,000 human genes into HEK293 cells, followed by culturing in the presence of arsenite (5 µM) for 48 hr. We subsequently searched for genes conferring cellular sensitivity against arsenite by suppressing gene expression. As a result, we identified ACOT9, a molecule within the superfamily of acyl-coenzyme A thioesterases (ACOTs), as the gene conferring the highest arsenite resistance to the cells by suppressing expression. Based on this result, we introduced two siRNAs with different sequences targeting ACOT9 (ACOT9 siRNA No. 1 or ACOT9 siRNA No. 2) into HEK293 cells to examine sensitivity to arsenite. We observed that cell cultures exposed to either ACOT9 siRNA showed higher resistance to arsenite, compared to cell exposed to the control siRNA (Fig. 1A). In addition, we observed that the level of ACOT9 mRNA decreased by ~70% following the introduction of either ACOT9 siRNA (Fig. 1B). Thus, ACOT9 may function to enhance the toxicity of arsenite.

Members of the ACOT family are known to regulate intracellular levels of CoA esters, free fatty acids and coenzyme A (CoASH) by hydrolyzing CoA esters,

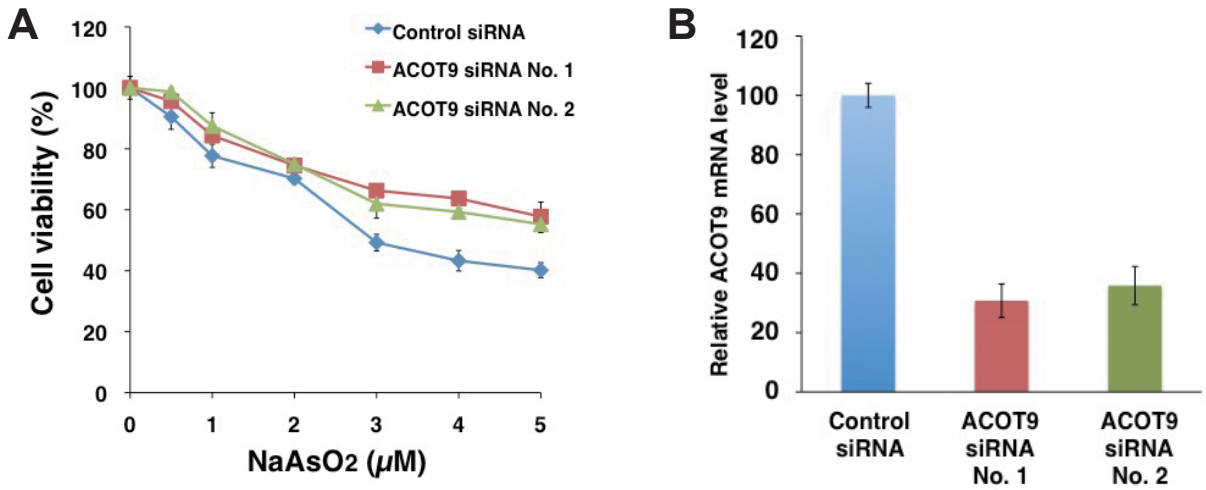

Fig. 1. Effect of ACOT9 knockdown on the sensitivity of HEK293 cells to arsenite. (A) HEK293 cells (1×10^4 cells/90 µL/well) transfected with ACOT9 siRNAs were seeded onto 96-well plates. After incubation for 48 hr, transfected cells were treated with the indicated concentrations of sodium arsenite for another 48 hr. Cell viability was quantified using an Alamar Blue assay. Points represent the mean results from three cultures; bars indicate standard deviation (S.D.). The absence of a bar indicates that the S.D. falls within the symbol. (B) mRNA levels of ACOT9, as measured using quantitative real-time PCR.

as well as have functions associated with lipid metabolism (Kirkby *et al*., 2010; Tillander *et al*., 2014). Many of ACOT9's functions are poorly understood, but myristoly-CoA (a molecule involved in the myristoylation of proteins) is known to be one of ACOT9's substrates (Poupon *et al*., 1999); the myristoylation of proteins has been reported to contribute to the induction of apoptosis (Vilas *et al*., 2006). Since the knockdown of ACOT9 inhibited the activation of caspase 3, an indicator of apoptosis, by arsenite exposure (data not shown), ACOT9 may enhance arsenite toxicity by promoting apoptosis induction following exposure to arsenite. In the future, by examining the relationship between ACOT9 and arsenite toxicity, we expect to obtain new findings that will further clarify the mechanism for developing arsenite toxicity.

Conflict of interest---- The authors declare that there is no conflict of interest.

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