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Toxicomics Report

Overexpression of palmitoyl transferase HIP14 confers resistance to methylmercury in SH-SY5Y human neuroblastoma cells

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ABSTRACT — We have previously identified Akr1 as one factor in the reduction of methylmercury toxicity in yeast, and reported that Akr1's palmitoyl transferase activity is necessary for reducing methylmercury toxicity. Palmitoylation transferases are highly conserved from yeast to humans. As such, we prepared SH-SY5Y neuroblastoma cells capable of overexpressing HIP14, a human homolog of Akr1, and investigated the cell's sensitivity to methylmercury. Our results showed that, compared with the control, the cells were resistant to methylmercury. Thus, we believe the palmitoyl transferase activity of HIP14 in humans can play an important role in the reduction of methylmercury toxicity.

Key words: Methylmercury, HIP14, Resistance, SH-SY5Y cells

INTRODUCTION

Methylmercury is an environmental toxicant, which damages the central nervous system and is the cause of Minamata disease (Mozaffarian and Rimm, 2006; Sanfeliu *et al.*, 2003). Although Minamata disease was first detected some 50 years ago, not much is known about the underlying mechanisms of methylmercury toxicity.

We previously used *Saccharomyces cerevisiae* as a eukaryotic model to examine the factors contributing to methylmercury toxicity (Hwang *et al.*, 2009, 2013 and 2014). Recently, we reported that Akr1, a palmitoyl transferase, is highly involved in the reduction of methylmercury toxicity in yeast and that palmitoyl transferase activity is necessary for reducing methylmercury toxicity (Hwang *et al*., 2016). Palmitoyl transferase is involved in endocytosis and apoptosis through the addition of palmitate to proteins, which then localize in the cellular and lysosome membranes (Linder and Deschenes, 2004; Smotrys and Linder, 2004). HIP14 is an Akr1 homolog in humans which has palmitoyl transferase activity in the brain (Singaraja *et al.*, 2002). In addition, it has also been reported that HIP14 is involved in the regulation of protein production and distribution in neurotransmitters (Huang *et al.*, 2004). In this study, we investigated the role of HIP14 in the reduction of methylmercury toxicity using human neuroblastoma cells.

MATERIALS AND METHODS

Materials and plasmids

Methylmercuric chloride was purchased from Sigma-Aldrich (St. Louis, MO, USA). The antibody to Myc epitope was obtained from MBL (Shiga, Japan). The antibody to GAPDH was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other reagents were of the highest grade available.

Complementary DNA (cDNA) was synthesized from total RNA prepared from SH-SY5Y cells using the Isogen reagent (Nippon gene, Tokyo, Japan) with an oligo primer (Gibco, Gaithersbug, MD, USA) and moloney murine leukemia virus reverse transcriptase (Gibco). The human HIP14 gene (NM_015336) was cloned by

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PCR with cDNA as the template. The following oligonucleotides were used as primers for amplification of HIP14: 5'-CAGCATGCAGCGGGAGGAGG-3' and 5'-gcggccgcGACACAAGCTGGTACCCAG-3'. The PCR product was ligated into the pTargeT vector (Promega, Madison, WI, USA) and the insertion was digested sequentially with restriction endonucleases (XhoI blunt and NotI). The resultant fragment was subcloned downstream of the EF-1α promoter into pEF4/Myc-His-A (Invitrogen, Carlsbad, CA, USA) that was then digested sequentially with restriction endonucleases (KpnI blunt and NotI).

Cell culture and transfection

Human neuroblastoma SH-SY5Y cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and supplemented with 10% fetal bovine serum, 0.3% *L*-glutamine and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin). The cells were grown at 37°C in a humidified incubator under an atmosphere of CO_2 (5%) and air (95%). For transient transfections, Lipofectamine 2000 (Invitrogen) was used according to the manufacturer's recommendations. To produce stable cell lines, the SH-SY5Y cells were transfected with the appropriate expression plasmids. After 48 hr, antibiotics for selection were added, and the cells were cultured for a further 2 weeks. Stable expression of recombinant proteins was confirmed by Western blotting.

Measurement of SH-SY5Y cell viability

Stable transfectants of the SH-SY5Y cells (1×10^4 cells) were plated on 96-well plates and cultured in 100 µL aliquots of medium. After incubation for 24 hr, methylmercuric chloride was added and the cells were cultured for a further 24 hr. Cell viability was determined by treating the cells with Alamar blue (Invitrogen) to a final concentration of 10% v/v, and the cells were incubated at 37°C for 3 hr. Cell fluorescence was measured using a Gemini XPS® microplate spectrofluorometer (Molecular Devices, Sunnyvale, CA, USA) with an excitation wavelength of 544 nm and emission at 590 nm.

Western blotting

SH-SY5Y cells expressing C-terminal-Myc-His6 tagged HIP14 (HIP14-Myc-His6) were washed on ice with cold PBS, and lysed in an RIPA buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM $MgCl_2$, 0.5% NP-40, and a protease inhibitor cocktail (Roche, Mannheim, Germany). The cell lysates were separated by SDS-polyacrylamide gel electrophoresis, transferred to an Immobilon-P membrane (Millipore, Bedford, MA, USA), and visualized using the relevant antibodies.

RESULTS AND DISCUSSION

We constructed SH-SY5Y human neuroblastoma cells capable of overexpressing HIP14-Myc-His5, a HIP14 fusion protein with a C-terminal Myc-His6 tag, and found that these cells showed greater methylmercury resistance when compared with the control group (Fig. 1). We then verified the HIP14-Myc-His6 overexpression using a Western blotting and anti-Myc antibodies (data not shown). From these results, we believe there is a possibility that HIP14, the Akr1 human homolog, is somehow involved in the reduction of methylmercury toxicity in neurons.

Palmitoyl transferase has a DHHC domain (aspartate-histidine-histidine-cysteine) in its structure that functions as the central active region; in humans there are at least 24 different proteins possessing this domain (Fukata and Fukata, 2010; Hornemann, 2015). It has been reported that many proteins that are involved in signal transduction between neurons are modified by palmitoylation (Fukata and Fukata, 2010; Prescott *et al.,* 2009). In particular, palmitoylation of PSD-95, a scaffold protein in

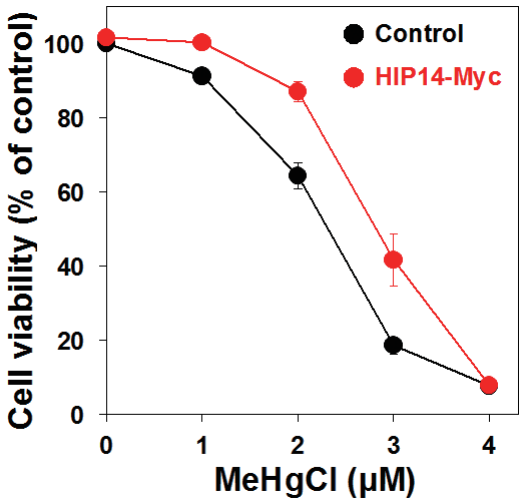

Fig. 1. Effect of HIP14 overexpression on the sensitivity of SH-SY5Y cells to methylmercury. SH-SY5Y cells (1×10^4 cells/well), transfected with pEF4 empty vector (Control) or pEF4-HIP14-Myc-His6 (HIP14-Myc), were seeded onto 96-well plates and cultured in 100 µL of DMEM. After incubation for 24 hr, the SH-SY5Y cells were treated with methylmercuric chloride (MeHgCl) for 24 hr. Cell viability was quantified using an Alamar blue assay. The error bars and data points represent the means and standard deviations from three independent experiments.

the postsynaptic membrane, is known to be necessary for the localization of various membrane proteins, such as the AMPA-type glutamate receptors (AMPA receptors) in the synapses (Opazo *et al.,* 2012). However, HIP14 is mainly distributed in the Golgi apparatus and was identified as a palmitoyl transferase that binds to the Huntingtin protein, the causative agent of Huntington's disease (Singaraja *et al.,* 2002). In addition, Butland *et al*. (2014) recently reported that reduced levels of palmitoylation of Huntingtin proteins could be highly related to the development of Huntington's disease. This suggests that HIP14 has a neuroprotective effect via palmitoylation of Huntingtin proteins. In future studies, we will attempt to elucidate the biological defense mechanism used to protect against methylmercury neurotoxicity. We will do this through the identification of HIP14-palmitoylated substrate proteins that are involved in the reduction of methylmercury toxicity.

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

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