

Letter

Small interfering RNA-mediated knockdown of the transcription factor TCF3 enhances sensitivity to methylmercury in mouse neural stem cells

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ABSTRACT — We have reported that four transcription factors (PAX4, PAX6, TCF3, and HMGA1) were markedly activated in the cerebellum of mice treated with methylmercury. In this study, to clarify the relationship between these transcription factors and methylmercury toxicity, siRNA targeting each of the four transcription factors were introduced individually into C17.2 mouse neural stem cells, and the sensitivity of the cells to methylmercury was investigated. Among the four transcription factors, knockdown of the gene for TCF3 increased the methylmercury sensitivity of C17.2 cells. Therefore, we suggest that TCF3 may have a protective effect against methylmercury toxicity.

Key words: Methylmercury, TCF3, Transcription factor

INTRODUCTION

Although methylmercury is known to be an environmental pollutant that shows selective toxicity in the central nervous system (Harada, 1995; Castoldi *et al.*, 2003), but the mechanism related to its toxicity are almost unknown. Recently, we used a protein/DNA array assay to comprehensively search for transcription factors activated in the cerebellum of mice treated with methylmercury, and identified four transcription factors (PAX4, PAX6, TCF3, and HMGA1) (Kim *et al.*, 2017). In this study, we investigated the relationship between these four transcription factors and methylmercury toxicity using C17.2 mouse neural stem cells.

MATERIALS AND METHODS

Cell culture

C17.2 mouse neural stem cells were cultured in Dulbecco's modified Eagle's medium (Nissui Pharmaceutical,

Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/L L-glutamine (Nacalai Tesque, Kyoto, Japan), and antibiotic (100 IU/mL penicillin and 100 mg/mL streptomycin) (Invitrogen, Carlsbad, CA, USA) in a humidified 5% CO₂ atmosphere at 37°C.

Measurement of sensitivity of C17.2 cells transfected with siRNA to methylmercury

Double-stranded siRNAs were purchased from Sigma-Aldrich (St. Louis, MO, USA) (Table 1). C17.2 cells were transfected with siRNAs using HiPerFect transfection reagent (Qiagen, Germantown, MD, USA) according to the manufacturer's protocol (Takahashi *et al.*, 2015). Briefly, double-stranded siRNA solution (10 nM final concentration) was added to HiPerFect transfection reagent and incubated for 10 min to allow formation of siRNA/HiPerFect complexes. After the incubation, a suspension of C17.2 cells was added to the siRNA/HiPerFect complexes. After a 15-min incubation at room temperature, C17.2 cells (1×10^4 cells) were diluted with cul-

Table 1. Knockdown efficacy of siRNA.

Gene	siRNA No.	Target sequence	Knockdown efficacy (% of control)
PAX4	#1	GCUUUGCACUGAAGGGCUU	88.6 ± 1.5
	#2	GUAUCUAAUGGCUGUGUGA	82.7 ± 6.3
PAX6	#1	GACUUCAGCUGAAGCGGAA	88.0 ± 0.6
	#2	CAGUGAAUGGGCGGAGUUA	90.3 ± 2.7
TCF3	#1	GCAUCAAGAGCAGCUCCCA	62.7 ± 2.4
	#2	CGGAUAAGGCGCAGACCAA	63.5 ± 4.1
HMGA1	#1	GUCAGAAAGAGCCCAGUGA	61.8 ± 3.0
	#2	CUCACUCUGACAAGGCCCA	71.5 ± 3.1

ture medium and seeded in 90 μ L aliquots/well in 96-well plates. Twenty-four hours after siRNA transfection, the C17.2 cells were treated with 10 μ L of methylmercuric chloride solution for 24 hr. Cell viability was measured by Alamar Blue assay (Biosource, Camarillo, CA, USA). Fluorescence was measured using a Gemini XPS microplate spectrofluorometer (Molecular Devices, Sunnyvale, CA, USA) (excitation wavelength 545 nm; emission wavelength 590 nm).

Confirmation of knockdown by quantitative real-time PCR

Total RNA was isolated from cells using an Isogen II kit (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. First-strand cDNA synthesis was carried out using a PrimeScript RT reagent kit (Takara, Shiga, Japan). Quantitative real-time PCR was performed using SYBR Premix EX Taq (Takara) and a Thermal Cycler Dice (Takara). The PCR primers used were: PAX4, 5'-ACCCTGTGACATTTACGGAG-3' (sense) and 5'-GTACTCGATTGATAGAGGAC-3' (antisense); PAX6, 5'-ACCAACGATAACATACCCAG-3' (sense) and 5'-CTGAAGTCGCATCTGAGCTT-3' (antisense); HMGA1, 5'-CAGGAAAAGGATGGGACTGA-3' (sense) and 5'-CTCCAGTTTCTTGCGTCTGC-3' (antisense); TCF3, 5'-TTGACCCTAGCCGGACATAC-3' (sense) and 5'-TGCCAACACTGGTGTCTCTC-3' (antisense); and GAPDH, 5'-ATCACCATCTTCCAGGAGCGA-3' (sense) and 5'-AGGGGCCATCCACAGTCTT-3' (antisense). Fold changes in mRNA levels were determined from standard curves after calibration of the assay.

RESULTS AND DISCUSSION

Two siRNAs were prepared for each of the four transcription factors of interest (PAX4, PAX6, TCF3, and

HMGA1), and each siRNA was transfected individually into C17.2 cells. The mRNA levels of the relevant transcription factors in siRNA-transduced cells were examined with quantitative real-time PCR, which confirmed that the expression levels of all the transcription factors were reduced by the introduction of the respective siRNA (Table 1). The methylmercury sensitivity of C17.2 cells with PAX4, PAX6, or HMGA1 knocked down was almost the same as that of controls. Conversely, C17.2 cells with TCF3 knocked down by either of the two siRNAs showed increased sensitivity to methylmercury (Fig. 1). Because methylmercury increases the DNA binding activity of TCF3 (Kim *et al.*, 2017), it is conceivable that TCF3 may regulate the expression of downstream genes in response to methylmercury exposure as an adaptive measure to reduce methylmercury toxicity. TCF3 belongs to the E protein family, and is known to be involved in the production of lymphocytes and associated cells (Guan *et al.*, 2016; Ikawa *et al.*, 2006); however, its function in the brain has not been studied extensively. Furthermore, the role of TCF3 in the mechanism of drug toxicity has not been studied. In the future, we expect that the role of TCF3 in the central nervous system will be clarified, and that new clues regarding the mechanism of methylmercury toxicity will be obtained.

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

Knockdown of TCF3 enhances methylmercury sensitivity

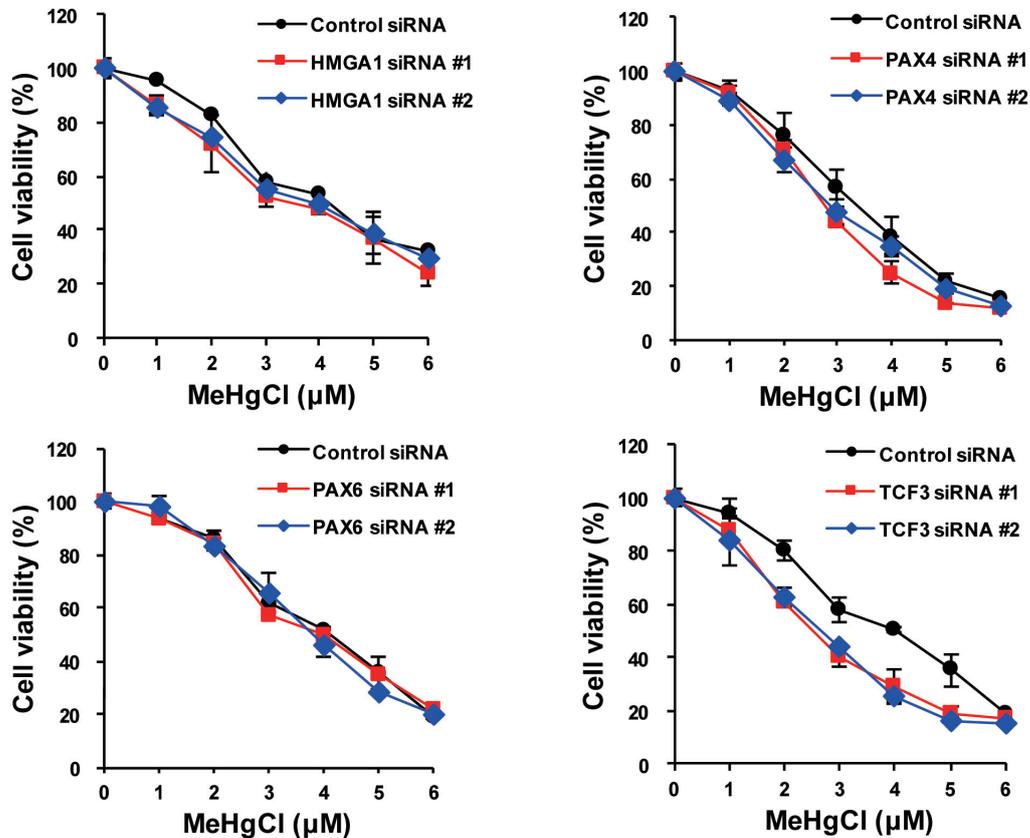


Fig. 1. Effects of PAX4, PAX6, TCF3 or HMG1 knockdown on the sensitivity of C17.2 cells to methylmercury. C17.2 cells (1×10^4 cells/90 μ L/well) transfected with siRNA were seeded onto 96-well plates. After incubation for 24 hr, transfected cells were treated with the indicated concentrations of methylmercuric chloride for 24 hr. Cell viability was quantified by Alamar Blue assay. Points represent the mean of results from three cultures; bars represent standard deviation. The absence of a bar indicates that the standard deviation falls within the symbol.

REFERENCES

- Castoldi, A.F., Coccini, T. and Manzo, L. (2003): Neurotoxic and molecular effects of methylmercury in humans. *Rev. Environ. Health*, **18**, 19-31.
- Guan, H., Xie, L., Wirth, T. and Ushmorov, A. (2016): Repression of TCF3/E2A contributes to Hodgkin lymphomagenesis. *Oncotarget*, **7**, 36854-36864.
- Harada, M. (1995): Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Crit. Rev. Toxicol.*, **25**, 1-24.
- Ikawa, T., Kawamoto, H., Goldrath, A.W. and Murre, C. (2006): E proteins and Notch signaling cooperate to promote T cell lineage specification and commitment. *J. Exp. Med.*, **203**, 1329-1342.
- Iwai-Shimada, M., Takahashi, T., Kim, M.S., Fujimura, M., Ito, H., Toyama, T., Naganuma, A. and Hwang, G.W. (2016): Methylmercury induces the expression of TNF- α selectively in the brain of mice. *Sci. Rep.*, **6**, 38294.
- Kim, M.S., Takahashi, T., Lee, J.Y., Hwang, G.W. and Naganuma, A. (2013): Global chemokine expression in methylmercury-treated mice: methylmercury induces brain-specific expression of CCL3 and CCL4. *J. Toxicol. Sci.*, **38**, 925-929.
- Kim, M.S., Takahashi, T., Lee, J.Y., Miura, N., Asanuma, M., Hwang, G.W. and Naganuma, A. (2017): Identification of transcription factors activated by methylmercury in mouse brain. *Fundam. Toxicol. Sci.*, **4**, 37-39.
- Takahashi, T., Kim, M.S., Saito, T., Lee, J.Y., Hwang, G.W. and Naganuma, A. (2013): Brain-specific induction of secretoglobulin 3A1 expression in mice treated with methylmercury. *J. Toxicol. Sci.*, **38**, 963-965.
- Takahashi, T., Kim, M.S., Hwang, G.W., Kuge, S. and Naganuma, A. (2015): Knockdown of Acyl-CoA thioesterase 9 gene expression by siRNA confers resistance to arsenite in HEK293 cells. *Fundam. Toxicol. Sci.*, **2**, 245-247.