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Toxicomics Report

Knockdown of TXNIP attenuates methylmercury toxicity in mouse neuronal C17.2 cells

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ABSTRACT — Methylmercury is an environmental pollutant that causes severe central nervous system damage. However, the mechanism involved in its toxicity remains unclear. In this study, expression of thioredoxin-interacting protein (TXNIP), which is involved in the regulation of intracellular redox status, was rapidly induced in mouse neuronal C17.2 cells in response to methylmercury exposure. In addition, C17.2 cells transfected with small interfering RNA against TXNIP mRNA showed greater resistance to methylmercury than control cells. These findings suggest that TXNIP is a novel factor involved in enhancing methylmercury toxicity and that methylmercury may cause cell death by inducing TXNIP expression.

Key words: Methylmercury, Toxicity, TXNIP

INTRODUCTION

Methylmercury is a pollutant that is ubiquitous in the environment. In the area around Minamata City, Kumamoto Prefecture, residents exposed to high concentrations of methylmercury have been observed to develop various neurological symptoms, including sensory, visual, and hearing impairments, as well as ataxia, and methylmercury toxicity has thus become a major social issue (Eto, 1997). In recent years, motor and mental development disorders have also been reported in children born to pregnant women who ingested relatively large amounts of methylmercury through seafood during pregnancy (Tatsuta *et al.*, 2017), and the health effects of methylmercury exposure are of global concern. However, details of the mechanism involved in methylmercury-associated neurotoxicity remain unclear.

We have previously identified and reported several novel factors involved in methylmercury toxicity as well as protective mechanisms against the toxicity (Yamashita *et al.*, 2024, 2025; Lee *et al.*, 2022). Using RNA sequencing, we investigated genes whose expression in mouse neuronal C17.2 cells was altered in response to methylmercury treatment and showed that expression of thioredoxin-interacting protein (TXNIP) was increased by methylmercury exposure. TXNIP belongs to the α-arrestin family and interacts with the antioxidant thioredoxin to suppress its function and control intracellular redox status (Tsubaki *et al.*, 2020). However, the role of TXNIP in methylmercury toxicity remains unclear. Therefore, in this study we investigated the relationship between methylmercury toxicity and TXNIP in C17.2 cells.

MATERIALS AND METHODS

Culture conditions

C17.2 cells were obtained from the European Collection of Cell Cultures. Cells were cultured in Dulbecco's

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Modified Eagle Medium (Shimadzu Diagnostics Corporation, Tokyo, Japan) supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and 2 mM L-glutamine (Nacalai Tesque, Kyoto, Japan) in a humidified atmosphere of 5% CO_2 at 37°C.

Quantitative polymerase chain reaction (qPCR)

Total RNA was isolated using ISOGEN II (Nippon Gene, Tokyo, Japan) and cDNA was synthesized from total RNA using a PrimeScript RT reagent kit with oligo dT primers (Takara, Shiga, Japan). qPCR was performed using SYBR Premix Ex Taq (Takara) and a LightCycler 96 System (Roche Diagnostics, Mannheim, Germany) with the following primers: TXNIP, F: 5′-TGCGTAGACTACTGGGTGAAGGC-3′, R: 5′-CAGACACTGGTGCCATTAGGTCA-3′; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), F: 5′-AACTTTGGCATTGTGGAAGG-3′, R: 5′-ACACATTGGGGGTAGGAACA-3′. The expression of each mRNA was determined using a relative standard curve method. TXNIP mRNA expression was normalized to that of GAPDH.

Small interfering RNA (siRNA) transfection and measurement of cell viability

Cells were seeded on 24-well plates and cultured for 24 hr. The indicated siRNA was then transfected using Lipofectamine RNAiMAX transfection reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. The cells were cultured for a further 24 hr for TXNIP knockdown and were then exposed to methylmercury at the indicated concentrations. After 24 hr, cell viability was determined using an alamarBlue assay (Invitrogen, Camarillo, CA, USA), according to the manufacturer's protocol. Fluorescence was measured using a SpectraMax iD5 (Molecular Devices, Sunnyvale, CA, USA) at excitation and emission wavelengths of 540 nm and 590 nm, respectively. All siRNAs were purchased from Sigma-Aldrich (St. Louis, MO, USA). Forward sequences for siRNA were as follows: TXNIP siRNA #1: 5′-GCUAUUGUGGCCCGACACAdTdT-3′, and TXNIP siRNA #2: 5′-GACACACUUACCUUGCCAAdTdT-3′. Negative control siRNAs were also obtained from Sigma-Aldrich.

Western blotting

Cells were lysed in a 2% sodium dodecyl sulfate (SDS) buffer, and the resulting lysates were incubated at 95°C for 5 min. Protein concentrations were measured using a DC Protein Assay Kit (Bio-Rad, Hercules, CA, USA). Cell lysates (approximately 7.5-10 µg) were separated by SDS–polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were blocked for 2 hr in 5% skim milk (Nacalai Tesque). Western blotting was performed with anti-TXNIP (K0205-3; MBL, Nagoya, Japan) and anti-GAPDH (015-25473; Fujifilm WAKO Pure Chemical Corp., Osaka, Japan) primary antibodies, followed by horseradish peroxidase-conjugated secondary antibodies (Dako A/S, Glostrup, Denmark). Chemiluminescent images were obtained using a ChemiDoc Touch Imaging System (Bio-Rad) and analyzed using Image Lab Software (version 5.2.1; Bio-Rad).

RESULTS AND DISCUSSION

First, we examined the effect of 4 µM methylmercury treatment on TXNIP gene expression in C17.2 cells by qPCR. TXNIP mRNA expression peaked at 2 hr and decreased at later treatment times (Fig. 1). It was also confirmed that methylmercury treatment did not have a significant impact on cell death (data not shown). These results indicate that the expression of TXNIP is temporarily increased prior to methylmercury-induced cell death.

Next, to clarify whether TXNIP is involved in methylmercury toxicity, siRNA against TXNIP mRNA was

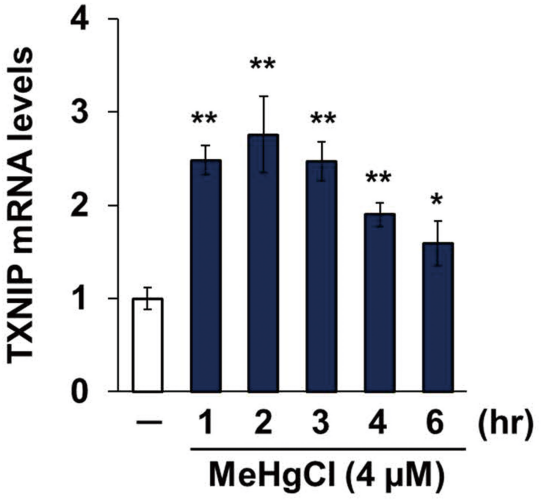

Fig. 1. Effects of methylmercury on TXNIP mRNA expression in C17.2 cells. Cells (5×10^4 cells/well) were seeded onto 24-well plates for 24 hr and exposed to 4 µM methylmercury chloride (MeHgCl) for the indicated times. TXNIP and GAPDH mRNA expression was measured and relative values normalized to those of GAPDH are shown. Data are presented as means ± standard deviations. $^{**}p < 0.01$, $^{*}p < 0.05$.

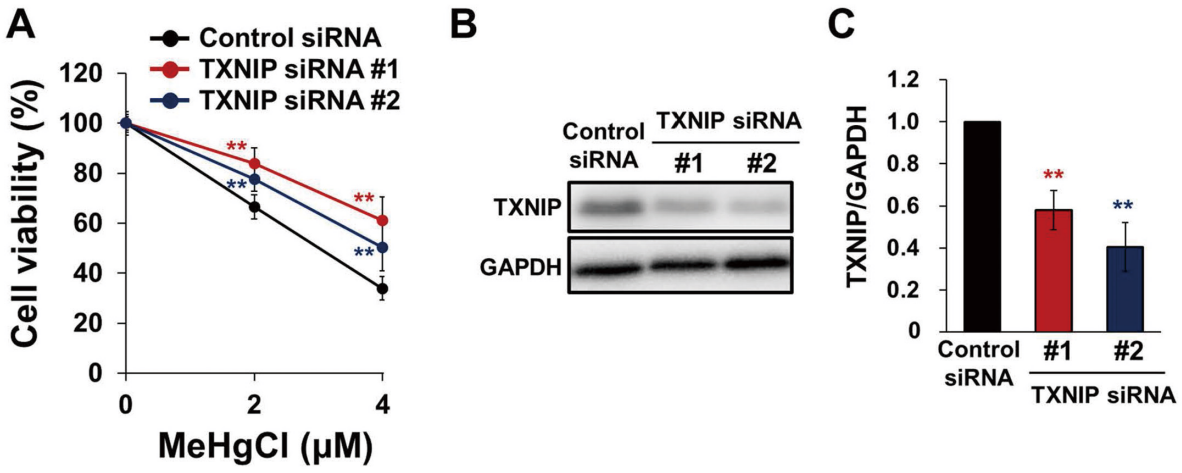

Fig. 2. Effects of TXNIP knockdown on methylmercury-induced apoptosis in C17.2 cells. Cells (2×10^4 cells/well) were seeded onto 24-well plates for 24 hr and transfected with control siRNA or TXNIP siRNA for a further 24 hr. Cells were then exposed to the indicated concentrations of methylmercury chloride (MeHgCl) for 24 hr. (A) Cell viability was measured using an alamarBlue assay. $^{**}p < 0.01$ vs control siRNA. (B, C) TXNIP and GAPDH protein expression was determined via Western blotting. $^{**}p < 0.01$ vs control siRNA.

introduced into C17.2 cells to suppress TXNIP expression. As a result, methylmercury-induced cell death was lower in C17.2 cells transfected with two types of siRNAs against TXNIP mRNA than in control cells (Fig. 2A). TXNIP protein expression was also decreased in both TXNIP siRNA-transfected cells (Fig. 2B, C). These results suggest that the increase in TXNIP expression induced by methylmercury exposure is involved in its toxicity.

It has been suggested that TXNIP expression, which is induced by lipopolysaccharide (Zhang *et al.*, 2022) and staurosporine (García-Hernández and Morán, 2023), may be involved in the induction of apoptosis in neurons. Apoptosis is known to be involved in methylmercury-induced cell death, and we have previously reported that methylmercury induces apoptosis in C17.2 cells (Toyama *et al.*, 2021; Sato *et al.*, 2020). These findings suggest that methylmercury may promote apoptosis-mediated cell death by increasing the expression of TXNIP. Future studies should therefore investigate the induction of TXNIP-mediated cell death by methylmercury.

The methylmercury-associated increase in TXNIP mRNA expression may involve transcriptional activation of the TXNIP gene. Indeed, we have also found that transcriptional inhibitors suppressed methylmercury-induced TXNIP mRNA expression (data not shown). It has been suggested that transcription factors activated by endoplasmic reticulum stress or glucose stimulation may be involved in the induction of TXNIP expression (Masutani, 2022), and that methylmercury may induce TXNIP expression by activating an unidentified transcription factor. Future studies should aim to identify transcription factors involved in the induction of TXNIP expression by methylmercury and investigate specific mechanisms.

In this study, we identified TXNIP as a novel factor involved methylmercury toxicity. Further clarification of the mechanism involved in TXNIP-mediated toxicity and induction of its expression by methylmercury is expected to lead to the establishment of preventive strategies against methylmercury toxicity.

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

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