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Transcription factor activation in rat primary astrocytes exposed to methylmercury

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ABSTRACT — To investigate the adaptive response of astrocytes to an environmental chemical, methylmercury (MeHg), we herein examined transcription factors activated in rat primary astrocytes treated with 10 μM MeHg for 6 hr using the Combo Protein/DNA Array. The activities of 38 transcription factors increased by 5-fold or greater in response to MeHg. The paired box family of transcription factors were strongly activated by MeHg exposure, which are considered to be activated downstream of NGF and BDNF upregulation induced by MeHg. Nrf-2 (an antioxidant response element) was also activated, which has been reported to act on MeHg detoxification.

Key words: Methylmercury, Astrocytes, Transcription factor network

INTRODUCTION

Methylmercury (MeHg) is an environmental pollutant that causes neuronal injury and neurological dysfunction. MeHg is known to accumulate especially in astrocytes in the brain (Aschner *et al.*, 1990), suggesting that astrocytes are a target of MeHg. In fact, gliosis, hyperplasia of activated astrocytes, was observed in the calcarine cortex and cerebellar granular layer of MeHg-intoxicated patients (Shiraki, 1979; Eto *et al.*, 1999). Astrocytes exposed to MeHg are reported to release interleukin-6 (IL-6) to protect neurons (Noguchi *et al.*, 2013). We recently demonstrated that the treatment of astrocytes with MeHg induced an upregulation of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which attenuated MeHg-elicited neurotoxicity (Takemoto *et al.*, 2015). Therefore, MeHg could activate a network of transcription factors in astrocytes to modulate the gene expression. In this study, we performed a global analysis of the transcription factors which are activated in astrocytes exposed to MeHg using the Combo Protein/DNA Array.

MATERIALS AND METHODS

Culture of rat primary astrocytes

All animal procedures were performed in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science, and Technology (Japan) and the Animal Care and Use Committee of Hiroshima University (Hiroshima, Japan). Pregnant Wistar rats were obtained from Kyudo (Kumamoto, Japan) and were maintained in a temperature-controlled animal facility with 12-hr light-dark cycles.

Cultures of primary astrocytes were prepared from the cerebral cortex of 1 to 2-day-old male Wister rats, as described previously (Ishihara *et al.*, 2015). Briefly, the cerebral cortex was treated with trypsin and DNase I to disperse the cells, which are then seeded on a poly-L-lysine coated plate. The purity of the astrocyte-enriched cultures was confirmed by staining with antibodies against the astrocyte-specific marker, glial fibrillary acidic protein (GFAP), and > 97% of cultured astrocytes showed immunoreactivity to GFAP (data not shown).

Protein/DNA Array

Rat primary astrocytes were treated with 10 μM MeHg for 6 hr, and then cells were collected. Nuclear extracts were prepared as described in our previous report (Ishihara and Shimamoto, 2006). The activity of 345 transcription factors was assessed using the Combo Protein/

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Table 1. Alterations in transcription factor activity in rat primary astrocytes treated with MeHg.

Transcription Factor	Description	Fold change
PAX-4	paired box 4	76.4
PAX-6	paired box 6	64.1
ISRE	interferon stimulated response element	48.7
L-IIIBP	L-III element-binding protein	38.1
MT-BOX	-	22.5
PAX-8	paired box 8	19.2
MUSF-1	mutant upstream stimulatory factor 1	17.2
CBF	core binding factor	15.8
Freac-2	forkhead box F2	15.7
PAX-3	paired box 3	15.5
LyF-1	IKAROS family zinc finger 1	15.2
HFH-3	forkhead box I1	14.3
CEA	carcinoembryonic antigen	13.0
E2F-1	E2F transcription factor 1	12.9
CEBP	CCAAT/enhancer binding protein	12.5
TFIID	transcription factor II D	11.9
HOXD-8/9/10	homeobox D 8/9/10	11.8
MEF-1	myocyte-specific enhancer-binding nuclear factor 1	11.3
HFH-2	hepatocyte nuclear factor 3/fkh homolog 2	11.1
NF-1	nuclear factor 1	10.6
CEF-2	cardiac enhancer factor 2	10.4
GATA-1	GATA binding protein 1	10.4
AP-3	activator protein 3	10.1
ARE	antioxidant response element	8.8
Pbx1	pre-B-cell leukemia homeobox 1	8.7
GATA-2	GATA binding protein 2	8.6
PARP	poly(ADP-ribose) polymerase	8.4
c-Rel	v-rel avian reticuloendotheliosis viral oncogene homolog	8.3
HMG	high mobility group protein	7.8
E47	-	7.3
Afxh/Foxo-4	forkhead box O4	7.3
GFI-1	growth factor independent 1	7.1
EGR-1	early growth response 1	6.7
SMAD-3/4	SMAD family member 3/4	6.6
FAST-1	forkhead box H1	6.3
HNF-1A	hepatocyte nuclear factor 1 homeobox A	6.2
Ets	-	6.0
Sp-1	specificity protein 1	5.0

Rat primary astrocytes were treated with 10 μM MeHg for 6 hr, and then changes in the activity of transcription factors were evaluated using the Combo Protein/DNA Array. Transcription factors whose activity increased by 5-fold or greater are listed.

DNA Array (Affymetrix, Santa Clara, CA, USA), according to the manufacturer's instructions. Biotin-labeled DNA-binding probes were incubated with the nuclear extracts. The probes specifically bound to the transcription factors were isolated using spin columns. The eluted, labeled probes were hybridized to a membrane containing an array of 345 transcription factor consensus binding sequences. The array was then washed and incubated with horseradish peroxidase-conjugated streptavidin and subsequently visualized using the ChemiDocTM XRS+ Imaging System (BIO-RAD, Hercules, CA, USA). Data were analyzed using the Image J software program and the relative activity of transcription factors was calculated.

RESULTS AND DISCUSSION

Treatment of rat primary astrocytes with 10 μM MeHg for 6 hr did not cause cell death (Takemoto *et al.*, 2015).

The Combo Protein/DNA Array analysis showed that the activity of 38 transcription factors increased by more than 5-fold following exposure to MeHg compared with untreated cells, as listed in Table 1. Interestingly, the paired box (PAX) family of transcription factors was strongly activated in astrocytes exposed to MeHg (Table 1). PAX is reported to be activated by NGF and BDNF (Kioussi and Gruss, 1994), and furthermore, we recently revealed that astrocytes treated with MeHg showed high expression levels of NGF and BDNF (Takemoto *et al.*, 2015). Therefore, PAX activation by MeHg might be an indirect effect of the increased expression of NGF and BDNF elicited by MeHg.

MeHg is well known to activate nuclear factor erythroid 2-related factor 2 (Nrf-2) by S-mercuration of the cysteine residue of kelch-like ECH-associated protein 1. According to our array analysis, the activation of an antioxidant response element, Nrf-2, was also detected in MeHg-treated astrocytes (Table 1). Nrf-2 is considered to attenuate MeHg-induced neurotoxicity due to an increased expression of anti-oxidative enzymes and/or phase II detoxification enzymes (Kumagai *et al.*, 2013).

As described above, MeHg increased the expressions of NGF and BDNF in rat primary astrocytes (Takemoto *et al.*, 2015). Activator protein 1 (AP-1) and cAMP response element binding protein (CREB) are well known to upregulate NGF and BDNF, respectively. However, the Combo Protein/DNA Array results indicated that treatment with MeHg did not affect the transcriptional activity of AP-1 and CREB. In addition, Noguchi *et al*. (2013) reported that the IL-6 expression also increased downstream of p38 phosphorylation by treatment with MeHg in astrocytes. Although nuclear factor-kappa B and AP-1 are involved in IL-6 transactivation (Vanden Berghe *et al.*, 1999), the activation of these molecules was not detected in astrocytes exposed to MeHg, indicating that the increased IL-6 expression in the classical pathway is independent of that activation. Collectively, astrocytes could respond to MeHg via a complex network of transcription factors to protect neurons from harmful stimuli.

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

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