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Microarray analysis of the liver in metallothionein-III null mice treated with cadmium

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ABSTRACT — In order to elucidate the effect of metallothionein (MT)-III on hepatic gene expression altered by cadmium (Cd), we examined gene expression patterns in the liver of MT-III null mice and wild-type mice after Cd injection using a DNA microarray containing 35,852 genes. In a comparison between Cd-injected MT-III null mice and Cd-injected wild-type mice, 9 genes were found to be up-regulated and 28 genes—including serum amyloid A1 (SAA-1) and SAA-2—were down-regulated.

Key words: MT-III, Cd, DNA microarray, Liver

INTRODUCTION

Cadmium (Cd) is a heavy metal that can induce various toxic effects such as hepatotoxicity, nephrotoxicity, osteotoxicity, and immunotoxicity. Metallothionein (MT) is a cysteine-rich low-molecular-weight protein with a high affinity for metals such as Cd and mercury (Klaassen *et al.*, 1999). MT has been identified in four major isoforms, and MT-I/II double knockout (MT-I/II null) mice and MT-III null mice have been established (Michalska and Choo, 1993; Masters *et al*., 1994; Erickson *et al.*, 1997). Using MT-I/II null mice, it has been previously reported that MT-I/II is an important protective factor against Cd toxicity (Satoh, 2007). On the other hand, our recent study has found that MT-III null mice are resistant to Cd hepatotoxicity (Honda *et al*., 2010). However, the molecular mechanism underlying this resistance remains unclear. In the present study, we examined hepatic gene expression in response to Cd between MT-III null mice and wild-type mice using DNA microarray analysis.

MATERIALS AND METHODS

Animals and treatment

MT-III null mice and wild-type mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and routinely bred in the laboratory animal facility of Aichi Gakuin University. MT-III null mice were of the 129/Sv strain. Age- and sex-matched 129/Sv mice were used as wild-type controls. All strains of mice were housed in cages in ventilated animal rooms at a controlled temperature of 23 ± 1°C with a relative humidity and maintained on standard laboratory chow and tap water *ad libitum*. They received humane care throughout the experiment according to the guidelines established by the Aichi Gakuin University.

Ten-twelve weeks old male MT-III null mice and wild-type mice were randomly assigned to experimental and control groups (n = 5). Each experimental group was administered a single subcutaneous injection of cadmium chloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at a dose of 20 μmol/kg. At 4 hr after the administration, the liver was removed from each mouse under diethyl ether anesthesia.

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Table 1. Changes in the hepatic gene expression of MT-III null mice and wild-type mice at 4 hr after Cd injection (20 μmol/kg)

Name of genes	Accession Number	Ratio
Ratio: MT-III null mice injected with Cd/Wild-type mice injected with Cd > 2.0		
aldolase 2, B isoform	NM_144903	2.69
DNA-damage inducible transcript 3	NM_007837	2.48
coenzyme Q10 homolog B (S. cerevisiae)	NM_001039710 NM_026424	2.44
cysteine-rich with EGF-like domains 2	NM_029720	2.36
interferon-related developmental regulator 1	NM_013562	2.30
glia maturation factor, beta	NM_022023	2.23
plastin 3 (T-isoform)	NM_145629	2.07
imprinted and ancient	NM_008378	2.05
cytochrome P450, family 2, subfamily c, polypeptide 38	NM_010002	2.01
Ratio: MT-III null mice injected with Cd/Wild-type mice injected with Cd < 0.5		
POU domain, class 3, transcription factor 1 (Pou3f1), mRNA	NM_011141	0.27
serum amyloid A1	NM_009117	0.31
DNA segment, human D4S114	NM_053078	0.43
death-associated kinase 2	NM_010019	0.43
Jun proto-oncogene related gene d	NM_010592	0.43
protein phosphatase 1, regulatory (inhibitor) subunit 14B	NM_008889	0.44
RIKEN cDNA 6430706D22 gene	NM_198652	0.44
rhomboid family 1 (Drosophila)	NM_010117	0.45
kidney expressed gene 1	NM_029550	0.45
BTB (POZ) domain containing 14A	NM_026495 NM_001037098	0.46
S100 calcium binding protein A8 (calgranulin A)	NM_013650	0.46
HIV-1 Rev binding protein-like	NM_178162	0.46
serum amyloid A2	NM_011314	0.46
voltage-dependent anion channel 1	NM_011694	0.46
interferon activated gene 202B	NM_008327	0.46
claudin 3	NM_009902	0.47
G protein-coupled receptor 98	NM_054053	0.47
vacuolar protein sorting 33B (yeast)	NM_178070	0.47
splicing factor, arginine/serine-rich 8	NM_172276	0.48
connective tissue growth factor	NM_010217	0.49
topoisomerase (DNA) III beta	NM_011624	0.49
PRP4 pre-mRNA processing factor 4 homolog B (yeast)	NM_013830	0.49
transgelin 2	NM_178598	0.49
notchless homolog 1 (Drosophila)	NM_145431	0.50
UTP6, small subunit (SSU) processome component, homolog (yeast)	NM_144826	0.50
motile sperm domain containing 3	NM_030037	0.50
predicted gene, EG434402	NM_001004191	0.50
late cornified envelope 5A	NM_025420	0.50

The genes for which the levels of expression changed more than 2-fold are listed here.

Extraction of RNA

Total RNA was extracted from the liver in the MT-III null mice and wild-type mice after Cd injection using the QuickGene RNA tissue kit S II (Fujifilm, Tokyo, Japan) according to the manufacture's protocol and stored at −80°C.

DNA microarray analysis

Total RNA (5 μg) was applied to an OpArray™ Mouse V4.0 slide, that had 35,852 genes registered (Operon Technologies, Alameda, CA, USA). We used a Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies Inc., Santa Clara, CA, USA) to synthesize complementary RNA (cRNA) from double-stranded cDNA as a template. A primer containing poly dT and T7 polymerase promoter was annealed to the poly A^+ RNA. Reverse transcriptase was then added to the reaction to synthesize the first and second strands of cDNA. Next, the double-stranded cDNA from wild-type mice or MT-III null mice was transcribed in the presence of cyanine (Cy) 3 or Cy5-labeled nucleotide, respectively. These two sets of fluorescence-labeled cRNA were mixed and hybridized to an OpArray™ slide for 16 hr at 42°C using a Lucidea SlidePro Hybridizer (GE Healthcare UK Ltd., Buckinghamshire, England). A fluorescent image of the OpArray slide was recorded with CRBIO (Hitachi Software Engineering, Tokyo, Japan). The digitized image data were processed with DNASIS Array software (Hitachi Software Engineering). After global normalization, the data were filtered to exclude genes with low expression levels. The ratios of the intensity of Cy5 (MT-III null mice injected with Cd) to that of Cy3 (wild-type mice injected with Cd) were then calculated and a difference of 2-fold change was applied to select up-regulated and down-regulated genes. Information on each gene on the slide was obtained from the National Center for Biotechnology Information (NCBI) database.

RESULTS AND DISCUSSION

The expression of the hepatic genes that were altered between the Cd-injected MT-III null mice and the Cd-injected wild-type mice is shown in Table 1. On comparing the Cd-injected MT-III null mice with the Cd-injected wild-type mice, 9 genes were found to be up-regulated and 28 genes were down-regulated in the MT-III null mice. Serum amyloid A1 (SAA-1) and SAA-2 were among the down-regulated genes; these are known to be regulated by inflammation-associated cytokines (Uhlar and Whitehead, 1999). Several studies have reported that SAA mRNA and protein are induced by Cd exposure (Yiangou and Papaconstantinou, 1993; Kayama *et al.*, 1995). MT-III null mice may therefore demonstrate a resistance to the inflammatory response after Cd injection. We consider that our findings would be useful for a better understanding of the involvement of MT-III in Cd hepatotoxicity.

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