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Abstract

Arsenicosis is diagnosed based on cutaneous symptoms such as pigmentation and hyperkeratosis. And it is reported that chronic arsenic exposure is associated with a variety of adverse health effects including cutaneous carcinoma, diabetes and visceral malignancy. But in the case of small amount of arsenic intake, it is occasionally difficult to establish the diagnosis of arsenicosis, and effects of arsenic exposure remains to be elucidated. This time, we constructed the mouse brain and liver differential expression library to clarify the molecular events caused by the intake of inorganic arsenic at low dose. We introduce the result of library screening.

Keywords: Arsenicosis mRNA expression, cDNA library, brain, liver

Introduction

Inorganic form of arsenic from natural sources in the environmental is commonly found in soil, air and water. It usually binds with carbon, iron sulfur and oxygen (World Bank, 2005a). But arsenic itself has toxicity to mammals including human-beings. And the upper limit of arsenic concentration in drinking water which is < 10 ppb is recommended by World Health Organization (WHO) in the guidelines for drinking water quality (WHO, 2011). Meanwhile, drinking groundwater containing arsenic over 10 ppb has been identified and reported all over the world including Europe, USA, and south-east Asia (World Bank, 2005a).

Focusing on Asia, arsenic contaminated groundwater from nature source has been recognized in Bangladesh, China, India, Nepal, Vietnam, Myanmar, Cambodia and Pakistan (Ahmad, Maharjan, Watanabe, & Ohtsuka, 2004; Chakraborti et al., 2002; Mukherjee et al., 2006; Ning et al., 2007; Singh, 2006). Actually, it is estimated that about 60 million people live in the area where groundwater is contaminated with arsenic. Moreover, patients suffering arsenicosis is estimated to be 700 thousand (World Bank, 2005b). Now, Exposure to arsenic derived from nature through contaminated groundwater is considered as one of the problems with public health.

Arsenic is classified into Group 1 carcinogen by International Agency for Research on Cancer (IARC). It increases the risk of various types of cancer such as pulmonary cancer, bladder cancer, lung cancer, squamous carcinoma and, renal cancer (ATSDR, 2007; Chakraborti et al., 2002; Miller, Schipper, Lee, Singer, & Waxman, 2002;

World Bank, 2005a).

Not only carcinogenesis but a variety of malignant health effects of arsenic exposure have been reported. It is indicated that arsenic exposure is associated with pigmentary changes of the skin (melanosis and leucomelanosis), hyperkeratosis, hypertension, diabetes mellitus, vascular diseases. Especially, early symptoms of chronic arsenic exposure are likely shown as pigmentation (such as spotted or diffused melanosis and leukoderma) and hyperkeratosis on the palms or body trunk. They depend on the amount of total uptake of arsenic (Howe et al., 2001; Rahman et al., 1999; Tseng et al., 2002, 2005; World Bank, 2005a).

Recently, epidemiological studies with school-aged children exposed to arsenic via drinking water indicated that arsenic exposure was associated with lower performance of intellectual functions (Calderón et al., 2001; Rosado et al., 2007). Urine and hair levels of arsenic are inversely correlated with intelligence quotient (IQ) (G. a. Wasserman et al., 2004; G. a Wasserman et al., 2007). In experimental study, alteration of behaviors such as alterations in motor behaviors, delay in acquisition and extinction of operant learning tasks and deficiencies of spatial learning paradigms were observed in rats exposed to inorganic arsenic.

The liver is one of the most important organs of arsenic metabolism, but most organs show arsenic methylating activity. In the liver, arsenic pentavalent (As^{V}) is reduced from pentavalent to trivalent (As^{III}). Subsequently, methyl group which is supplied mainly by S-adenosylmethionine (SAM) is added to As^{III}. Although the exact sequence of events has not been elucidated, methylarsonic acid (MMA) and dimethylarsinic acid (DMA) were predominantly biosynthesized at the end of arsenic metabolism.

In the encephalon, mechanism of arsenic neurotoxicity partly comes to be clear. For example, it was reported that that monomethylarsonous acid (MMA^{III}) caused the impairment of the excitatory CA1 synapse via its action on postsynaptic glutamatergic receptors. Meanwhile, a part of arsenic toxicity to the liver which plays an important role in arsenic metabolism is also uncovered. Arsenic can increase lipid peroxidation and suppress antioxidant defense in liver, and the liver turns out to be susceptible to oxidative stress (Kitchin and Ahmad,2003; Guha Mazumder,2005; Vizcaya-Ruiz et al.,2009). But the molecular mechanism underlying these findings still remains to be elucidated. Especially, the alteration of gene expression via arsenic exposure is left to be focused on.

In our study, the screening for altered mRNA expression was conducted so that we could identify the genes whose expression increased or decreased in response to arsenic exposure especially in the brain and liver.

Result and Discussion

Tester and Driver cDNA pairs

In this study, subtractive hybridization method was used as described before. mRNA whose expression increased specifically in tester sample was concentrated via this method, and it enabled the construction of cDNA libraries which reflected the expression pattern of mRNA. First of all, mRNA was extracted from liver and brain which were derived from control mice and arsenic exposed mice. Double strand cDNA were synthesized using these four types of mRNA, and four combinations of tester and driver cDNA were tested shown in Table 1. cDNA derived from brain of arsenic exposed mice was selected as tester, and cDNA from that of control mice as driver. In the same manner, cDNA from liver of arsenic exposed mice was chosen to be tester and cDNA from that of control mice driver. And reverse library was also established vice versa.

Table 1 Combination of tester and driver cDNA

	Liver Library	Reverse Liver Library	Brain Library	Reverse Brain Library
Tester	Liver, Arsenic exposed	Liver, Control	Brain, Arsenic exposed	Brain, Control
Driver	Liver, Control	Liver, Arsenic exposed	Brain, Control	Brain, Arsenic exposed

Subtraction efficiency

The subtraction efficiency was evaluated by comparing the amount of G3PDH cDNA contained in pre- and post-subtracted cDNA via polymerase Chain Reaction and subsequent electrophoresis. Template cDNA concentration was adjusted to be equal and PCR was carried out with G3PDH specific primer pair. Then electrophoresis of PCR products was performed using 2% agarose/ethidium bromide gel, and the efficiency of subtractive hybridization was confirmed (data not shows).

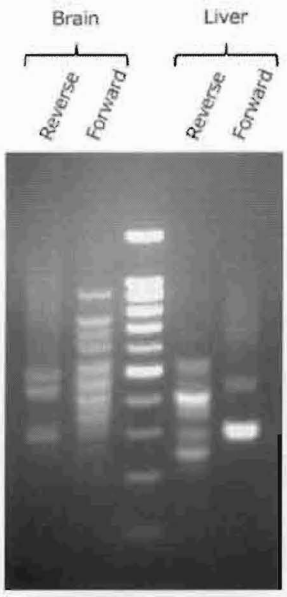

Fig. 1
Electrophoretic profile
of 2nd PCR Products

Result of Subtraction

After second PCR which is the last step of subtractive hybridization, the electrophoresis of PCR products was conducted with 2% agarose / ethidium bromide gel shown in Fig. 1. It showed that DNA fragment patterns of Brain Library were quite different from that of Reverse Brain Library, and it also suggested that the patterns of Liver Library were not equal to that of Reverse Liver Library. This result indicated that low dose intake of inorganic arsenic altered mRNA expression at least in brain and liver. In order to identify such mRNA, sequence analysis was conducted. In each library, 16 DNA fragments were subsequently sequenced so far, and totally 17 genes which were up-regulated in response to arsenic exposure were identified.

Discussion

In previous studies, a variety of adverse health effects of arsenic exposure has been reported; carcinogenesis, reproductive effects, effects to encephalon. Nevertheless, the underlying mechanism of malignant health effect caused by arsenic exposure remains to be elucidated. In this study, expression cDNA libraries were constructed and the screening of these libraries was conducted in order to identify the genes whose expression altered in response to arsenic exposure. As a result, 17 genes were obtained which were expected to be up-regulated or down-regulated. 8 genes out of 17 genes were obtained from brain libraries, and the rest of 9 genes were from liver libraries. Further study is now going on such as quantitative PCR, northern blot analysis and protein quantification so as to clarify the effect of the molecular events caused by low dose arsenic intake on biological organisms.