MRP5b/SMRP mRNA is Highly Expressed in Metallothionein- Deficient Mouse Liver

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To investigate whether multidrug resistance protein 5 (MRP5) functions as a xenobiotic detoxification factor, we measured MRP mRNA expression levels in metallothionein (MT)-I, II knockout mouse, and the results showed that MRP5b/SMRP, a splicing variant of MRP5, was highly expressed in the liver of MT-I, -II null mice.

Key words —— metallothionein, multidrug resistance protein, multidrug resistance protein 5

INTRODUCTION

Metallothionein (MT) is a small protein that plays a role in protecting against the toxic effects of heavy metals and xenobiotics as well as in drug resistance and free radical scavenging,[1–4] and MT knockout mice exhibit hypersensitivity to heavy metals, such as a cadmium[5,6] and mercury.[7] MT is known to be one of the factors involved in resistance to cisplatin, and Sato *et al*. reported that MT knockout mice are more sensitive to cisplatin than normal mice.[8]

We previously cloned multidrug resistance protein 5 (MRP5)/SMRP, a member of the MRP family, from cisplatin-resistant lung cancer cell line PC-14/CDDP, and the expression levels of MRP5/SMRP have been found to be enhanced by cisplatin in lung cancer patients.[9–11] Since McAleer *et al*. reported that MRP5 confers resistance to cadmium and antimony,[12] we speculated that MRP5 plays a role in heavy metal detoxification in MT-I, II knockout mice instead of MT, and in this study we investigated MRP5 mRNA expression levels in the tissues of MT-I, II knockout mice.

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MATERIALS AND METHODS

Animals —— Homozygous MT-I and MT-II knockout mice (MT-null), and the corresponding wild-type (parental strain), 129/sv mice, were used.[13] The animals were housed in a temperature-controlled and light-controlled animal room.

RNA Isolation and RT-PCR Analysis —— Total RNA was extracted from the mouse tissues with ISOGEN reagent according to the protocol provided by the manufacturer (Nippongene, Toyama, Japan). cDNA synthesis and the polymerase chain reaction were carried out by using a RevaTra Dash RT-PCR kit according to the protocol provided by manufacturer (TOYOBO, Tokyo, Japan). The amplification primer pairs were: MRP1, MRP2, MRP3, MRP4, MRP5 and G3PDH, 5′-CTG GGT TTG TTC CGG ATC AAT G-3′ and 5′-TGA CAC AAA GCC CTT TAG GTG A-3′; 5′-CTG CCT CTT CAG AAT CTT AG-3′ and 5′-CCC AAG TTG CAG GCC AGC CAC-3′; 5′-CCA TGA CTC TTT GCC TGT TCC G-3′ and 5′-TCA TCT AGG CAA GTC CCG CAT C-3′; 5′-AGT GCA AGT AGC GCC CAC CC-3′ and 5′-AGC TCC TCG TCC GTG TGC TC-3′; 5′-GTG GTG ACC TTC TCT GTT CA-3′ and 5′-ATA CTG GAG TGG GAG GAG TC-3′; 5′-ACC ACA GTC CAT GCC ATC AC-3′ and 5′-TCC ACA CCC TGT TGC TGT A-3′. PCR was cycled 30 times (98°C for 30 sec, 60°C 2 sec, 74°C 30 sec) and followed by incubation at 74°C for 2 min. The PCR products were separated by electrophoresis in 8% TBE polyacrylamide gel or 1% TAE agarose gel, stained with ethidium bromide, and visualized under an ultraviolet transilluminator.

RESULTS AND DISCUSSION

To investigate MRP5 function as a detoxification factor for xenobiotics, we investigated the mRNA expression levels of MRP5 in the MT-I, II

knockout mouse by performing RT-PCR analysis of brain, lung, heart, liver, and kidney tissue from three animals each (Fig. 1). The MRP5 expression patterns were similar to those we obtained in humans, and no marked difference in MRP5 expression levels between any of the tissues of the MT-null and wild-type mice was observed. However, the MRP5 expression pattern in the liver differed slightly from its pattern of expression in the other tissues. To clarify the expression pattern in the liver, we performed a second PCR analysis (Fig. 2), and the results showed that the long fragment was the major product in the MT-I, II knockout mouse. We previously reported that MRP5 has two variants, MRP5a/MRP5 and MRP5b/SMRP, and since MRP5b was preferentially expressed in liver and skeletal muscle, we speculated that the long fragment expressed in MT-null mouse liver was MRP5b/SMRP. We recently found that adriamycin inducees both MRP5a/MRP5 and MRP5b/SMRP in lung cancer cell lines and that these MRP5s are over-expressed in adriamycin-resistant cell lines,[14] and in this study we found that SMRP is expressed in the liver of the MT-I, -II null mice. Although the physiological function of MRP5s remains unknown, MRP5b/SMRP may play a role in the detoxification of xenobiotics by these mice instead of MT. We also investigated the expression levels of MRP family members other than MRP5 in the tissues obtained from the same mice. The results showed no differences in expression levels of MRP1, 2, 3, and 4 between the brain, lung, heart, and kidney tissues of the MT-null mice and wild type mice, and the tissues distribution patterns were similar to those in humans. Typical expression patterns are shown in Fig. 3.

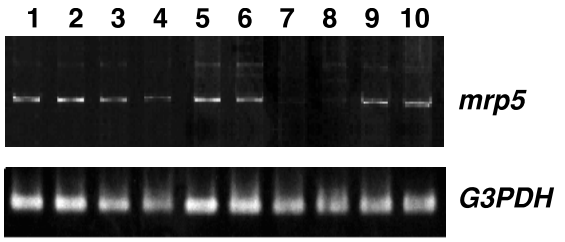

Fig. 1. Expression Analysis of MRP5 in MT-Null Mouse Tissue

PCR was performed using primer sets for MRP5 and G3PDH. The total RNA templates were prepared from MT-null mouse tissue (lane 2, 4, 6, 8, 10) and wild-type mouse tissue (lane 1, 3, 5, 7, 9). The bands of G3PDH provide an indication of the amount of total RNA loaded in each lane. Lane 1, 2: brain; lane 3, 4: lung; lane 5, 6: heart; lane 7, 8: liver; lane 9, 10: kidney.

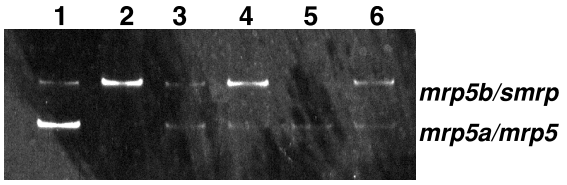

Fig. 2. Expression Analysis of MRP5 Variant in MT-Null Mouse Liver

First PCR products obtained from MT-null mouse liver (lane 2, 4, 6) and wild-type mouse liver (lane 1, 3, 5) were used as a template. Other experimental conditions were same as those of Fig. 1.

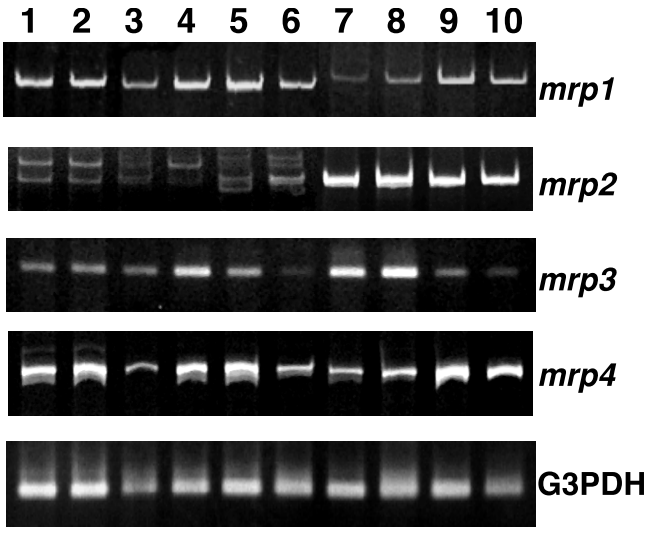

Fig. 3. Expressi on Pattern of MRPs on MT-Null Mouse Tissues

MRP1, -2, -3, -4 were detected by RT-PCR. The total RNA templates were prepared from MT-null mouse tissue (lane 2, 4, 6, 8, 10) and wild-type mouse tissue (lane 1, 3, 5, 7, 9). The bands of G3PDH provide an indication of the amount of total RNA loaded in each lane. Lane 1, 2: brain; lane 3, 4: lung; lane 5, 6: heart; lane 7, 8: liver; lane 9, 10: kidney.

In conclusion, our results suggest that the expression level of SMRP may be increased by MT deficiency in the liver and that SMRP or MRP5 may contribute to heavy metal detoxification in the liver of MT-null mice instead of MT.

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