Xenobiotics transporter MRP1 (Multidrug Resistance Protein 1) cloned from bovine - Comparison of its expression, structure and functions with human and mouse MRP1

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Abstract

Previously, we cloned bovine MRP1 cDNA from cow's mammary gland, as a candidate for genes that make influence on quality of milk. In this paper, we investigated the tissue distribution of MRP1 mRNA in bovine, and compared the amino acid sequence of bovine MRP1 with those of human and mouse MRP1. Northern blotting showed that the expression of bovine MRP1 mRNA occurs in a wide range of tissues, but little in liver and brain, as described in the case of human and mouse MRP1. Bovine MRP1 consists of 1530 amino acids with 91% and 87% identity with the human and mouse ortholog, respectively. The comparison of amino acid sequences and functions among bovine, human and mouse MRP1s revealed that the poor ability to confer resistance to doxorubicin of bovine MRP1 is likely due to the fact that the amino acid in bovine MRP1 corresponding to Glu^{1089} in human MRP1 is Gln^{1088} as in the mouse ortholog. Our results support the notion that this amino acid residue of MRP1 plays an important role in the activity of this transporter, especially in the ability to confer resistance to doxorubicin.

1. Introduction

Cancer cells often acquire resistance to multiple anti-cancer drugs having no structural and functional similarities. This phenomenon is termed multidrug resistance (MDR), and is a major obstacle to cancer chemotherapy. MDR is frequently associated with the expression of multidrug resistance-associated protein 1 (MRP1, Fig. 1). MRP1 is a members of the ATP-binding cassette

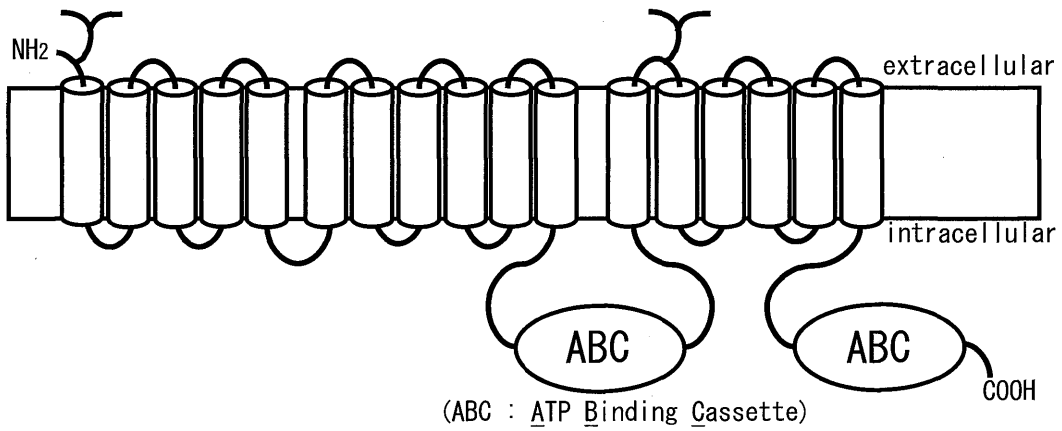

Fig. 1 Schematic representation of the putative structure of MRP1

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(ABC) family of membrane transport proteins, functions as an energy-dependent efflux pump that extrudes many kinds of xenobiotics out of cells[1-3]. MRP1 can transport a wide range of natural chemotherapeutic agents including anthracyclines, *Vinca* alkaloids, and epipodophyllotoxins[4-6]. MRP1 can also transport negatively charged conjugated hydrophilic compounds, such as glutathione conjugates[7-13], and certain arsenic- and antimony-centered oxyanions[6].

By several studies using MRP1 knock out mice, it has been suggested that one of the physiological functions of MRP1 is to protect important tissues or organs in the host's body against xenobiotics. For examples, MRP1 expressing in the basal membrane of Sertoli cells functions as the blood-testis barrier[14], and that expressing in choroids plexus epithelia functions as the blood-cerebrospinal-fluid barrier[15,16]. Recently, we found that there was lower transfer of antimony, one of the substrates of MRP1, from blood to milk in lactating mice (manuscript in preparation). These results suggest that MRP1 protein expressed in the mammary gland would play a role in protecting milk against xenobiotics.

Thus, we cloned bovine MRP1 cDNA from mammary gland of lactating cow, as a candidate for genes that make influence on quality of milk[17]. Bovine MRP1 consists of 1530 amino acids with 91% identity with the human ortholog. The cells expressing bovine MRP1 showed resistance to several drugs, to which human MRP1 can confer resistance. However, bovine MRP1 conferred much lower resistance of doxorubicin (a member of anthracyclines), and higher resistance to VP16 (a member of epipodophyllotoxins) than human MRP1. These results suggested that in substrate specificity, bovine MRP1 differs from human MRP1, despite a high degree of similarity in amino acid.

In this paper, we investigated the tissue distribution of MRP1 mRNA in bovine, and compared the amino acid sequence of bovine MRP1 with those of human and mouse MRP1.

2. Materials and Methods

2.1 Materials

Mammary gland of lactating cow (Holstein) was kindly supplied by Dr. Shu Hashimoto, Snow Brand Milk Products Co., Ltd. Other tissues of cow were generously provided by Dr. Yoshikazu Nagao, Utsunomiya University.

2.2 Northern blot analysis

Poly(A)$^+$ RNA from various tissues of cow was obtained as described above. Two micrograms of poly(A)$^+$ RNA was separated on 1% agarose gel containing formaldehyde[18]. Following transfer to Nitroplus 2000 (Micron Separations Inc.), the filter was hybridized in 6×SSC, 5×Denhardt's solution, 0.5% SDS, 4 mM EDTA, 100 μg/ml salmon sperm DNA, and ^{32}P-labeled cDNA fragments for 16 hr at 65°C. The filter was washed in 0.5×SSC at 65°C and then exposed to x-ray film.

3. Results and Discussion

Northern blot analysis was done in order to investigate the tissue distribution of MRP1 in bovine (Fig. 2). It was shown that bovine MRP1 is expressed in heart, spleen, lung, kidney, skeletal muscle and mammary gland, and with low expression in brain, and liver. This tissue profile of expression resembles that reported for human and mouse, suggesting that the physiological functions of MRP1 in bovine would be similar to those in human and mouse[1,19].

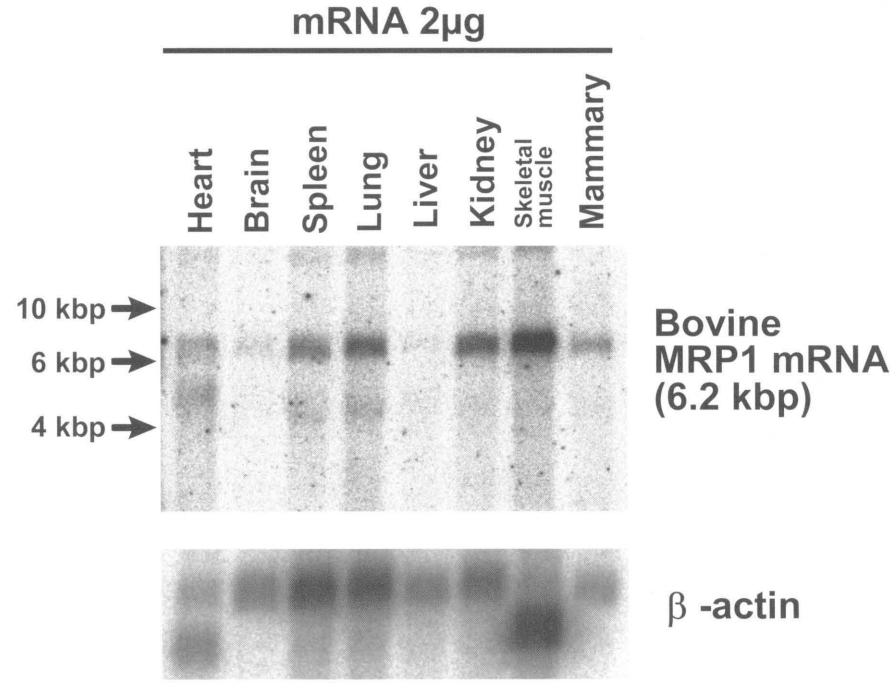

Fig. 2 Northern blot analyses of MRP1 mRNA expression in various bovine tissues. 2μg of poly(A)$^+$ RNAs from the indicated bovine tissues were separated on a 1% agarose gel containing formaldehyde, and blotted to nitrocellulose membrane. The blot was hybridized with a ^{32}P-labeled 0.6-kb fragment of bovine MRP1 cDNA.

The deduced amino acid sequence of bovine MRP1 was compared with those of human and mouse orthologs (Fig.3). The amino acid sequence of bovine MRP1 shows 91% and 87% identity with that of the human and mouse ortholog, respectively. However, we showed that the substrate specificity of bovine MRP1 differs from that of human MRP1 in spite of a high degree of similarity in amino acid[17]. Especially, the cells expressing bovine MRP1 showed much lower resistance to doxorubicin than those expressing human MRP1. The low ability to confer doxorubicin resistance of bovine MRP1 is similar to that of mouse MRP1. The mutational analysis of mouse and human MRP1 revealed that the amino acid residue at position 1089 of human MRP1 (corresponding to position 1086 in mouse MRP1) is critical for the ability of the protein to confer drug resistance particularly to doxorubicin[20]. The mutation of Gln^{1086} in mouse MRP1 to Glu markedly increased the resistance to doxorubicin, while the mutation of Glu^{1089} in human MRP1 to Gln decreased the resistance. The amino acid residue of bovine MRP1 corresponding to this amino acid residue is Gln at position 1088, suggesting that the poor ability to confer resistance to doxorubicin of bovine MRP1 is likely due to the fact that the amino acid in bovine MRP1 corresponding to Glu^{1089} in human MRP1 is Gln^{1088} as in the mouse ortholog. Our results support the notion that this amino acid residue of MRP1 plays an important role in the activity of this transporter, especially in the ability to confer resistance to doxorubicin[20].

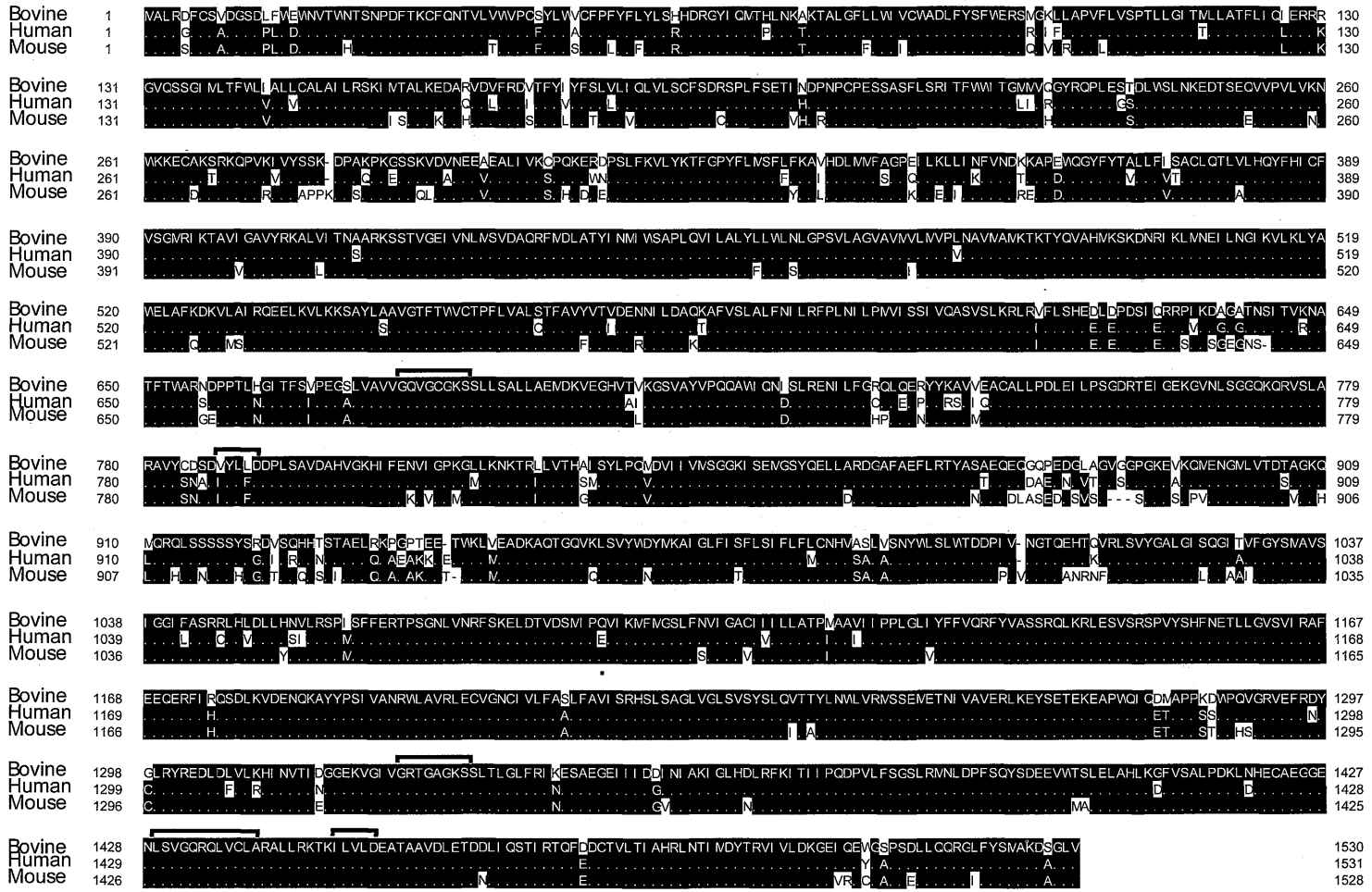

Fig.3 Alignment of the predicted amino acid sequence of bovine, human and human MRP1s. Alignment was performed with GENETYX software. Conserved amino acids are shaded. Bracketed amino acids are the motifs conserved among ATP Binding Casstee (ABC) domains of ABC transprters. A: Walker A motif, B: Walker B motif, and C: ABC signature (active transport signature). The amino acid residue Glu1088, corresponding to Glu1089 in human MRP1, is indicated by arrow.

Some of non-conserved amino acids between bovine and human orthologs, such as Gln^{1088} in bovine and Glu^{1089} in human, are supposed to be important in deciding the substrate specificity of MRP1. The higher resistance to VP16 as well as lower resistance of doxorubicin of cells expressing bovine MRP1 than those expressing the human ortholog might be caused by such amino acid residues. The mutational analysis of the non-conserved amino acid residues between MRP1 orthologs might help to clarify the domains involved in recognition and/or transport of the substrates of this transporter.

Acknowledgement

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和文抄録

ウシより単離した有害物質排出タンパク質 MRP1 –ヒトおよびマウス MRP1 との発現、構造および機能の比較解析

田口善智、佐伯和弘

MRP1 は、抗ガン剤や重金属などの有害物質を、細胞内から外へ排出するポンプタンパク質である。我々は、母体内の有害物質が乳汁中に移行することを防ぐことにより、牛乳の性質に影響を与える可能性のある遺伝子として、授乳中のウシ（ホルスタイン）乳腺より MRP1 の cDNA を単離した。本研究では、ウシの各組織における MRP1 mRNA の発現を解析すると同時に、ウシ MRP1 とヒトおよびマウス MRP1 のアミノ酸配列と機能の比較を行った。その結果、MRP1 は広い範囲のウシ組織において発現しており、特に、その発現量は、肺、腎臓、骨格筋で高く、脳、肝臓では比較的低いことがわかった。また、ウシ MRP1 の 1088 番目のアミノ酸残基が、この輸送タンパク質の抗ガン剤アドリアマイシンの輸送能力と関連しており、ウシ MRP1 のアドリアマイシンの輸送能力が低いのは、このアミノ酸が、アドリアマイシンの輸送能の高いヒト MRP1 と同じグルタミン酸ではなく、その輸送能の低いマウス MRP1 と同様にグルタミンであるためであることが示唆された。