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Preparation and Characterization of a Murine Monoclonal Antibody (MDR3M) Reactive with *mdr*3 Gene Product

Isamu Sugawara,[1,3] Akio Hirata,[1] Kazumitsu Ueda[2] and Shinji Itoyama[1]

[1]*Department of Pathology, Saitama Medical Center, Saitama Medical School, 1981 Kamoda, Kawagoe, Saitama 350 and* [2]*Department of Agriculture, Kyoto University, Oiwake-cho, Kitashirakawa, Sakyo-ku, Kyoto 606*

A murine monoclonal antibody (MDR3M) (isotype: IgM) reactive with *mdr*3 gene product was generated by immunizing mice with *mdr*3-specific peptide (H_2N-[12]WRPTSAEGDFELGISSKQKRK-KTKTVKMI[41]G-COOH) and hybridizing the primed mouse splenic B cells with X63-Ag8,6.5.3 mouse plasmacytoma cells. MDR3M did not cross-react with *mdr*1 gene product. This monoclonal antibody may be useful for analyzing the role of *mdr*3 gene product in cells and tissues.

Key words: *mdr*3 gene product — Monoclonal antibody — *mdr*3 peptide

The *mdr*1 gene product is known to be present in the membranes of cancer cells resistant to adriamycin, *Vinca* alkaloids, colchicine and other anti-cancer agent.[1-3] Mammalian P-glycoproteins (P-GPs) are encoded by small gene families. *mdr*1 and *mdr*3 (also called *mdr*2) are present in humans,[4,5] while hamsters and mice each have three P-GPs.[6-10] The sequence of human *mdr*3 cDNA has been determined, and the protein it encodes has a deduced molecular weight of 140 kDa.[5] It has been reported that stable *mdr*3-experssing clones do not display multidrug resistance, unlike those expressing the *mdr*1 gene product.[11] There is only indirect evidence that human *mdr*3 gene product might confer daunorubicin resistance on PLL cells.[12,13] Thus, the function of *mdr*3 gene product still remains unknown. As a first step toward clarifying the nature of this protein, we attempted to prepare a murine monoclonal antibody (MAb) against *mdr*3 gene product. The *mdr*3-specific peptide, which did not cross-react with *mdr*1 gene product, was synthesized on a peptide synthesizer (Excel, Milligen Biosearch Inc.). It consisted of 30 amino acids (H_2N-[12]WRPTSAEGDFELGISSKQKRKKTKTVKMI[41]G-COOH).[5] The peptide was injected five times with adjuvant into BALB/c mice at a concentration of 100 μg/100 μl at two-week intervals. Subsequently, the mouse splenic cells were fused with murine plasmacytoma cells (X63-Ag8,6.5.3).[14] After screening and cloning, a hybridoma clone of IgM isotype was selected. The isotype was determined by the Ouchterlony method.[15]

First, we checked the immunoreactivity of MAb MDR-3M with *mdr*3-expressing Hep G2 cells by immunocytochemistry (ABC-GO method).[16] Hep G2 is a hepatocellular carcinoma cell line established by Aden *et al.*[17] Reverse transcription-polymerase chain reaction (PCR) was carried out as described previously to see whether the Hep G2 cells produce *mdr*1 and *mdr*3 gene products or not, using *mdr*1-specific primer sets (the sizes of the amplified bands were 596 and 742 bp) and an *mdr*3-specific primer set (the size of the amplified band was 772 bp). β_2-Microglobulin gene-specific primer set was used as an internal control (the size of the amplified band was 261 bp. Thirty cycles of PCR were carried out using a thermal cycler, denaturing at 94°C for 1 min, followed by annealing and extension at 65°C for 5 min each.[18] The Hep G2 cells were found to express both *mdr*1 and *mdr*3 mRNA (Fig. 1). The Hep G2 cells were fixed with 4% paraformaldehyde for 60 min and, after being washed, were cytospun. For absorption experiments, 100 μg of an *mdr*3-specific peptide was treated with 10 μg of MAb MDR3M at room temperature for 30 min. Then the mixture was centrifuged at 10,000 rpm for 30 min and the supernatant was used as the absorbed antibody. MAb MDR3M reacted with Hep G2 cells strongly, whereas the MAb absorbed with *mdr*3-specific peptide lost its immunoreactivity with Hep G2 cells (Fig. 2). We further examined the immunoreactivity of the MAb with KB-3-1 and a KB-G2 transfectant. KB-3-1 is a subline of KB cells (an epidermoid carcinoma in the mouth of an adult male Caucasian) and is highly sensitive to multiple anti-cancer drugs.[1] KB-3-1 possesses hardly any *mdr*1 or *mdr*3 gene product. The KB-G2 transfectant was prepared by one of the present authors (K.U.) by introducing the *mdr*1 gene into KB-3-1 cells; these KB-G2 cells expressed P-GP intensely, as assessed by Western blotting and RT-PCR. As shown in Fig. 2, the MAb did not react with the KB-G2 cells, but C219, which reacts with *mdr*1 gene product prepared in Dr. V. Ling's laboratory, did react with them strongly.[19]

[3] To whom requests for reprints should be addressed.

We carried out Western blotting to evaluate the native *mdr*3 gene product recognized by MAb MDR3M.[20, 21] As shown in Fig. 3, the molecular weight of the native *mdr*3 gene product was estimated to be about 170 kDa. Since the deduced molecular weight of the *mdr*3 gene product is about 140 kDa and it is underglycosylated, the native *mdr*3 gene product may be a glycoprotein.[5] The peptide we synthesized on a peptide synthesizer was part of the intracytoplasmic domain. Thus, MAb MDR3M recognized the intracytoplasmic portion of *mdr*3-expressing Hep G2 cells, as confirmed by immunoelectron microscopy (data not shown).

In summary, it is evident that our monoclonal antibody reacts with *mdr*3 gene product. The MAb should be a useful tool for helping to clarify the role of *mdr*3 gene product both *in vitro* and *in vivo*.

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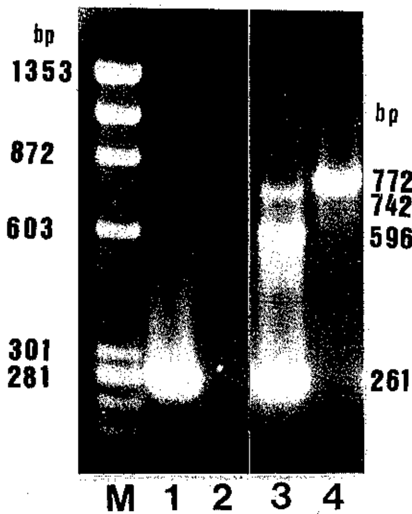

Fig. 1. Expression of *mdr*1 and *mdr*3 mRNA by RT-PCR. *mdr*1-specific amplified bands (596 and 742 bp) are recognized in Hep G2 cells (lane 3), but hardly in KB-3-1 cells (lane 1). An *mdr*3-specific amplified band (772 bp) is recognized in Hep G2 cells (lane 4), but not in KB-3-1 cells (lane 2). M; DNA size marker.

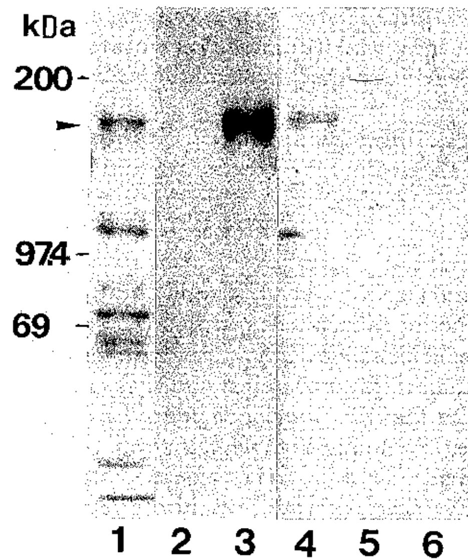

Fig. 3. Immunoblotting of Hep G2, KB-3-1 and KB-G2 cells reacted with MAb C219 and MAb MDR3M. Lane 1, Hep G2; lane 2, KB-3-1; lane 3, KB-G2; lane 4, Hep G2; lane 5, KB-3-1; lane 6, KB-G2. Hep G2 cells possess both *mdr*1 and *mdr*3 gene product (►) (lanes 1 and 4). KB-G2 possesses only *mdr*1 gene product (lane 3). Neither *mdr*1 gene product nor *mdr*3 gene product was recognized in KB-3-1 cells (lanes 2 and 5).

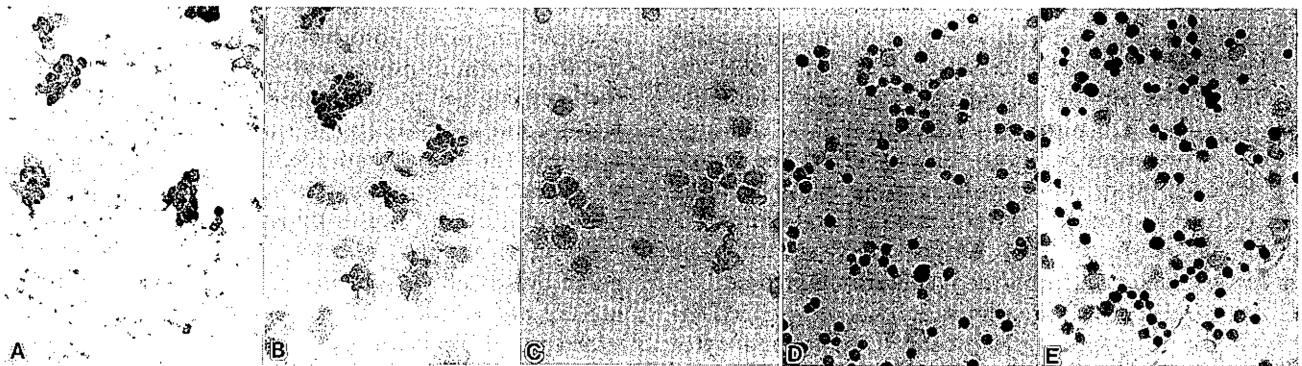

Fig. 2. Expression of *mdr*3 gene product in various cells. ABC-glucose oxidase (GO) method. ×100. A, Hep G2 cells immunostained with MAb MDR3M; B, Hep G2 cells immunostained with MAb MDR3M absorbed with *mdr* 3-specific peptide; C, KB-3-1 cells immunostained with MAb MDR3M; D, KB-G2 cells immunostained with MAb MDR3M; E, KB-G2 cells immunostained with MAb C219. Only Hep G2 cells are immunostained positively with MAb MDR3M and MAb C219 (A).

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