Elevated Expression of P-Glycoprotein in Kidney and Urinary Bladder Cancers

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Department of Pathology, the Institute of Medical Science, the University of Tokyo, Tokyo 108, *Department of Pathology, Saitama Medical Center, Kawagoe 350, †Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo 170, ‡the Institute of Applied Microbiology, the University of Tokyo, Tokyo 113, ∥Department of Urology, the Tokyo Kosei-nenkin Hospital, Tokyo 162, and Departments of §Urology and ¶Pathology, the University of Akita, Akita 010

Moriyama, M., Sugawara, I., Hamada, H., Tsuruo, T., Kato, T., Sato, K., Hikage, T., Watanuki, T. and Mori, S. Elevated Expression of P-Glycoprotein in Kidney and Urinary Bladder Cancers. Tohoku J. Exp. Med., 1991, 164 (3), 191-201 — A monoclonal antibody, MRK16, recognizing specifically an epitope of P-glycoprotein (P-GP), a highly active efflux transporter of chemotherapeutic agents, was used to determine the degree of expression of P-GP in the normal human kidney and urinary bladder, and in kidney and urinary bladder cancers. P-glycoprotein was localized in the microvilli of the epithelial cells of the proximal renal tubules by immunoelectron microscopy, and detected immunohistochemically in 6 of 20 untreated kidney cancers and 11 of 31 untreated urinary bladder cancers. Some of the cancerous tissues were further examined with regard to P-GP expression by immunoprecipitation. In urinary bladder cancers, the degree of P-GP expression seemed to be somewhat correlated with tumor grading. These results indicate that our method to detect the degree of expression of P-GP by MRK16 may be applicable for the diagnosis of clinical multidrug resistant urinary cancers. ——— p-glycoprotein; multidrug-resistance; MDR-1; urinary tract cancer

As chemotherapy becomes an important means of cancer treatment, resistance to multiple anti-cancer drugs has become a major clinical problem to be overcome. It has been reported that a 170 to 180 kDa glycoprotein (P-glycoprotein) is a
highly active efflux transporter of chemotherapeutic agents (Juliano and Ling 1976; Tsuruo 1988). Up to present, P-glycoprotein (P-GP) is found in various normal human tissues such as adult adrenal cortices, proximal renal tubules, placenta, biliary ductules, pancreatic ductules, epithelia of jejunum and colon, and secretory epithelia of uterus gravida (Thiebaut et al. 1987; Arceci et al. 1988; Sugawara et al. 1988a, b). The P-GP is also expressed in benign adrenocortical tumors, malignant lymphoma, leukemia, ovarian cancer, as revealed by monoclonal antibodies, MRK16 and C219 recognizing different epitopes of P-GP (Bell et al. 1985; Kartner et al. 1985; Hamada and Tsuruo 1986; Ma et al. 1987; Tsuruo et al. 1987; Ito et al. 1989; Sugawara et al. 1989a, b). However, it is not yet known whether multidrug-resistance gene (MDR1) gene product (P-GP) recognized by MRK16 monoclonal antibody (Mab) is expressed at protein level in the kidney cancer and urinary bladder cancer or not, though MDR1 is expressed at mRNA level (Fojo et al. 1987; Kakehi et al. 1988; Chin et al. 1989; Goldstein et al. 1989). This paper first reports the expression of P-GP recognized by a monoclonal antibody, MRK16, in the kidney cancer and the urinary bladder cancer.

**MATERIALS AND METHODS**

Twenty human kidney cancerous tissues, two human kidney benign tumor tissues, one human kidney and 31 urinary bladder cancerous tissues, either surgically excised or transurethrally biopsied on patients who had not been given any anti-cancer drugs, were obtained at Tokyo Koseinenkin Hospital, Tokyo University Hospital, and Akita University Hospital. The fetal kidney (24week gestation) was obtained from an autopsy performed within one hour of death on a patient with heart disease at Saitama Medical Center, Saitama Medical School. The tissues for frozen section studies were immediately snap-frozen in liquid nitrogen and stored at -70°C until sectioning. The histological characteristics of the cancers are summarized in Table 1. Adriamycin-resistant K562 (K562/ADM) as a positive control cell line containing P-GP was provided by one of the authors (Tsuruo et al. 1986).

*Separation and characterization of a monoclonal antibody, MRK16*

The methods used for antigen preparation, immunization, cell fusion, cloning, and serological characterization of monoclonal antibody, MRK16 have been previously described in detail (Hamada and Tsuruo 1986).

*Immunohistochemistry*

Avidin-biotin-peroxidase (ABC-PO) method and avidin-biotin-glucose oxidase (ABC-GO) method were used for the frozen sections according to an instruction sheet issued by Vector Lab., Burlingame, CA, USA. Briefly, the frozen sections (5 μg in thickness) were cut with a cryostat, fixed with 4% paraformaldehyde for 30 min, and washed with phosphate-buffered saline (PBS). Then, the preparations were reacted with MRK16 (10 μg/ml), or non-immune mouse serum (10 μg/ml) as a negative control at room temperature (RT) for 30 min after a 30-min blocking with 100-fold diluted horse serum in PBS. After three washings with PBS, 50 μl aliquots of 1 : 100-diluted biotinylated goat anti-mouse IgGs

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*Fig. 1.* Immunostaining of the normal fetal kidney (A, ABC-PO method), adult kidney (B, ABC-GO method), and adrenal (C, ABC-PO method) with MRK16. ×400.
were applied for 30 min at RT. Thereafter, 100-fold diluted ABC-PO or ABC-GO solution was applied. After coloration with $H_2O_2$ and diaminobenzidine or with $\beta$-glucose, nitroblue tetrazolium, and phenazine methosulfate, the preparations were examined with a light microscope (Olympus Optical Co., Tokyo) (Hsu et al. 1981; Sugawara et al. 1988c).

**Immunoelectron microscopy**

To examine the localization of the P-GP recognized by MRK16 Mab, an immunoelectron microscopical study was performed. The frozen sections (5 $\mu$m thick) were cut in a cryostat, placed on a glass slide and were first fixed with periodate-lysine-paraformaldehyde for 45 min at 4°C. Thereafter, they were treated with MRK16 Mab (10 $\mu$g/ml) at 4°C for 12 hr. After being washed carefully with PBS 3 times, 50 $\mu$l of peroxidase-labeled rabbit anti-mouse IgGs (DAKO PATTS, Copenhagen, Denmark) were applied for 60 min at RT. They were fixed with 2.5% glutaraldehyde in PBS for 20 min after frequent washing with PBS. Thereafter, 500 $\mu$l of 3, 3-diaminobenzidine-$H_2O_2$ solution (2.5 mg of 3, 3-diaminobenzidine and 10 $\mu$l of $H_2O_2$ in PBS) were added for 10 min for coloration. After three washings with PBS, they were postfixed with 1% osmic acid solution for 30 min and embedded in Epon 812. The ultrathin sections were prepared using an ultramicrotome (Ultracut E; Reichert-Jung Co., Wien, Austria). These were then stained with uranylacetate and examined with an electron microscope (Model 100C; JEOL, Tokyo).

**RESULTS**

**Localization of MRK16-defined P-GP in the normal kidney and urinary bladder**

As shown in Fig. 1, MRK16 reacted with the epithelial cells of both the fetal (24 week gestation) and adult proximal renal tubules. The adrenal cortex used as a positive control also possessed P-GP. The reactivity of MRK16 with the epithelial cells of the fetal proximal renal tubules was almost the same as that with the epithelial cells of the adult proximal renal tubules. As shown in Fig. 2, P-GP was immunoelectronmicroscopically localized in the microvilli of the epithelial cells of the renal proximal tubules. However, transitional epithelia of the urinary bladder did not possess P-GP as assessed by immunocytochemistry and immunoprecipitation (data not shown).

**Table 1. Reactivities of MRK16 Mab with kidney and urinary bladder cancers and the histological types**

<table>
<thead>
<tr>
<th>Reactivity</th>
<th>TCC* (bladder)</th>
<th>RCC (Clear cell+ granular cell)</th>
<th>Renal angiomyolipoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
<td>G3</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

*TCC, transitional cell carcinoma; RCC, renal cell carcinoma.
Fig. 2. Ultrastructural localization of P-glycoprotein in the epithelial cells of the proximal renal tubules by MRK16 Mab. Note that P-glycoprotein (arrow) is present in the microvilli. \( \times 10,000 \). (A) MRK16 Mab (10 \( \mu \)g/ml); (B) non-immune mouse serum.
Reactivities of MRK16 with kidney and urinary bladder cancers

As shown in Fig. 3, the MRK16 reacted with K562/ADM cells as a positive control, 6 of 20 renal cell carcinomas, and 11 of 31 transitional cell carcinomas of the bladder, while all of the renal mesenchymal tumors (angio(myo)lipoma) stained negative. Of the renal cell carcinomas, granular cells stained positively more strongly than clear cells (Fig. 3). This finding was confirmed by serially cut cryostat sections. Table 1 shows that 25% of grade 1 urinary bladder tumors showed P-GP reactivity, whereas about 44% of grade 3 urinary bladder tumors showed P-GP reactivity. The MRK16-positive cells were distributed diffusely but heterogeneously (Fig. 4).

DISCUSSION

Our present study revealed that: (a) P-GP recognized by MRK16 Mab was expressed strongly in normal renal proximal tubules and was expressed frequently but moderately in untreated renal cell carcinomas. (b) P-GP was not expressed in normal transitional epithelia of urinary bladder, but sometimes expressed in untreated transitional cell carcinomas. (c) We succeeded in detecting P-GP in surgically excised cancerous tissues.

From these results, there are four points to be commented upon. First, we found that the P-GP was expressed at a high level in the normal renal proximal tubules, and renal cell carcinoma. This may indicate that MRK16-positive renal cell carcinoma is derived from the proximal renal tubules and that the normal renal proximal tubules excrete unknown metabolites through P-GP. At the ultrastructural level, microvilli of a renal proximal tubule was immunostained positive. There is a tendency that in the light microscopic immunohistochemistry, P-GP is distributed diffusely in the proximal tubules because the specimen for electron microscopy is about 70 nm thick, whereas the preparation for the light microscopy is 5 μm thick. As we could not obtain very fresh samples of renal cell carcinomas showing P-GP reactivity, we could not utilize immunoelectron microscopy.

Second, P-GP was not recognized in normal transitional epithelia as far as we examined by immunohistochemistry (ABC-PO method) and immunoprecipitation (data not shown), though Chen et al. have shown that MDR1 mRNA is expressed in normal urinary bladder. Several transitional cell carcinomas have possessed P-GP. Twenty-five percent of grade 1 bladder tumors showed P-GP reactivity, while about 44% of grade 3 bladder tumors showed P-GP reactivity. However, their sampling is too small to determine whether there is a correlation between the
Fig. 4. Immunostaining of the transitional cell carcinoma with MRK16. (A) papillary type, G1; ABC-GO method with MRK16. (B) papillary type, G1; ABC-GO method with non-immune mouse serum. (C) nonpapillary type, G3; ABC-PO method with MRK16. (D) nonpapillary type, G3; ABC-PO method with non-immune mouse serum. ×400.
grade and the immunoreactivity or not. The expression of P-GP may be easily
induced in transitional cell carcinomas as a result of mutation. Further studies
should be required to elucidate the mechanism of P-GP induction.

Third, our immunohistochemical method to detect P-GP will be more useful
and economical, from the point of clinical application, than the slot blot hybridiza-
tion for MDR1 mRNA by Shen et al. (1988). It is advisable, however, to use
that another immunohistochemical method, ABC-GO method, for confirmation of
P-GP was found by ABC-PO method. ABC-GO method is also preferable to ABC-PO method when many endogenous peroxidase-containing granulocytes are present in surgical specimens.

Finally, we evaluated the frequency of the P-GP expression in the untreated kidney and urinary bladder cancers. In the future, the evaluation of the P-GP expression in the cancers induced by anti-cancer drugs will be required. Furthermore, it should be investigated whether the degree of the P-GP expression correlated with clinical multidrug resistance or not. From our present data, it is suggested that MRK16 Mab may have two potentially useful clinical applications. One is that the Mab may be useful for detecting the degree of multidrug resistance in vitro and eventually choosing anti-cancer drugs. The other is that the Mab, either by itself or in combination with toxins or radioisotopes, may be used for the selective ex vivo killing of cancer cells containing high levels of P-GP. MRK16 Mab can be used in vivo with caution, because there is the possibility that MRK16 Mab may damage endothelial cells by binding to them (Sugawara et al. 1990).

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References


