

Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues

(immunohistochemistry/liver/adrenal/kidney/cancer chemotherapy)

FRANZ THIEBAUT*, TAKASHI TSURUO†, HIROFUMI HAMADA†, MICHAEL M. GOTTESMAN*, IRA PASTAN*, AND MARK C. WILLINGHAM*

*Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and †Division of Experimental Chemotherapy, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan

Contributed by Ira Pastan, July 23, 1987

ABSTRACT Monoclonal antibody MRK16 was used to determine the location of P-glycoprotein, the product of the multidrug-resistance gene (*MDR1*), in normal human tissues. The protein was found to be concentrated in a small number of specific sites. Most tissues examined revealed very little P-glycoprotein. However, certain cell types in liver, pancreas, kidney, colon, and jejunum showed specific localization of P-glycoprotein. In liver, P-glycoprotein was found exclusively on the biliary canalicular front of hepatocytes and on the apical surface of epithelial cells in small biliary ductules. In pancreas, P-glycoprotein was found on the apical surface of the epithelial cells of small ductules but not larger pancreatic ducts. In kidney, P-glycoprotein was found concentrated on the apical surface of epithelial cells of the proximal tubules. Colon and jejunum both showed high levels of P-glycoprotein on the apical surfaces of superficial columnar epithelial cells. Adrenal gland showed high levels of P-glycoprotein diffusely distributed on the surface of cells in both the cortex and medulla. These results suggest that the protein has a role in the normal secretion of metabolites and certain anti-cancer drugs into bile, urine, and directly into the lumen of the gastrointestinal tract.

A major problem in the chemotherapy of cancer is the cross-resistance of some primary and many recurrent human tumors to multiple chemotherapeutic drugs (1). Such multidrug-resistant tumor cells have been shown to have a highly active efflux mechanism for chemotherapeutic drugs, which prevents accumulation of these drugs in the cytoplasm of multidrug-resistant cells (2, 3). Recently, this type of multidrug resistance has been shown to be due to the product of a gene (*MDR1*) that confers the multidrug-resistant phenotype (4-8). The sequences of the human *MDR1* gene and two rodent genes have been determined and shown to encode a polypeptide that has multiple membrane-spanning hydrophobic domains and two nucleotide binding sites that are structurally related to subunits of active transport pumps present in bacteria (9-12). The mature form of this transmembrane glycoprotein is 170 kDa and is termed P170 or P-glycoprotein (13-15). A monoclonal antibody to P-glycoprotein with a determinant present on the external surface of human multidrug-resistant cells has been isolated (16). This antibody, termed MRK16, has been used to localize P-glycoprotein in the plasma membrane of human multidrug-resistant cells in culture (17). The location and structure of P-glycoprotein are consistent with its proposed role as an energy-dependent efflux pump.

The expression of the human *MDR1* gene has been studied using RNA extracted from normal tissues and tumor samples (18). The gene was found to be expressed at high levels in

human adrenal, liver, colon, and kidney and in many tumors derived from these organs. Since cancers of these organs are often drug resistant, it is assumed that the *MDR1* gene product, P-glycoprotein, functions to transport chemotherapeutic drugs out of these tumor cells. Its function in normal cells is not clear, but it could participate in the removal by excretion of cytotoxic chemicals found in the diet. If this hypothesis is correct, P-glycoprotein should be present on the apical or luminal surface of secretory cells in the organs in which the gene is expressed. The current study was designed to determine the location of P-glycoprotein in normal tissues. To do this, fresh frozen human tissues were cryostat-sectioned and mounted on coverslips followed by drying and formaldehyde fixation. P-glycoprotein was localized using peroxidase immunocytochemistry with MRK16. In agreement with RNA analyses, P-glycoprotein was mainly found in adrenal, kidney, colon, small intestine, and liver. Its location within cells of these organs is consistent with its proposed role as a drug-transport protein.

MATERIALS AND METHODS

Tissues. Human tissues from autopsy or surgical material were obtained and frozen within 2 hr. The fresh nature of the tissues was found to be critical for the detection of P-glycoprotein. Some of the tissues were obtained from the Tissue Procurement Service, University of Alabama at Birmingham, and some were obtained from the National Institutes of Health Clinical Center. Freshly frozen normal human liver samples were the generous gift of Irwin Arias (Tufts Medical School, Boston). Human tissues examined for P-glycoprotein localization included liver, kidney, adrenal, colon, jejunum, stomach, lung, cerebral cortex, cerebellum, spinal cord, salivary gland, ovary, uterus, spleen, skin, and placenta.

The MRK16-Reactive Determinant of P-glycoprotein. MRK16 mouse monoclonal antibody was prepared as described (16). Preliminary experiments with a multidrug-resistant cell line (KB-C4) (17) using immunofluorescence indicated that the MRK16-reactive determinant was detectable as an external epitope in living cells and in cells primarily fixed in 3.7% formaldehyde. Cells fixed in formaldehyde and then exposed to Triton X-100 showed a weakened reaction, but cells fixed in formaldehyde and exposed to saponin-containing solutions remained strongly reactive (see below; see also Fig. 3). Cells primarily fixed in acetone showed very weak localization, and cells fixed in ethanol showed no localization. For these reasons, formaldehyde fixation of cryostat sections was used for the immunohistochemistry as described below. The loss of reactivity after ethanol is not thought to represent extraction of P-glycoprotein but rather a loss of reactivity with the MRK16-reactive determinant, since in parallel experiments, cells fixed in acetone and

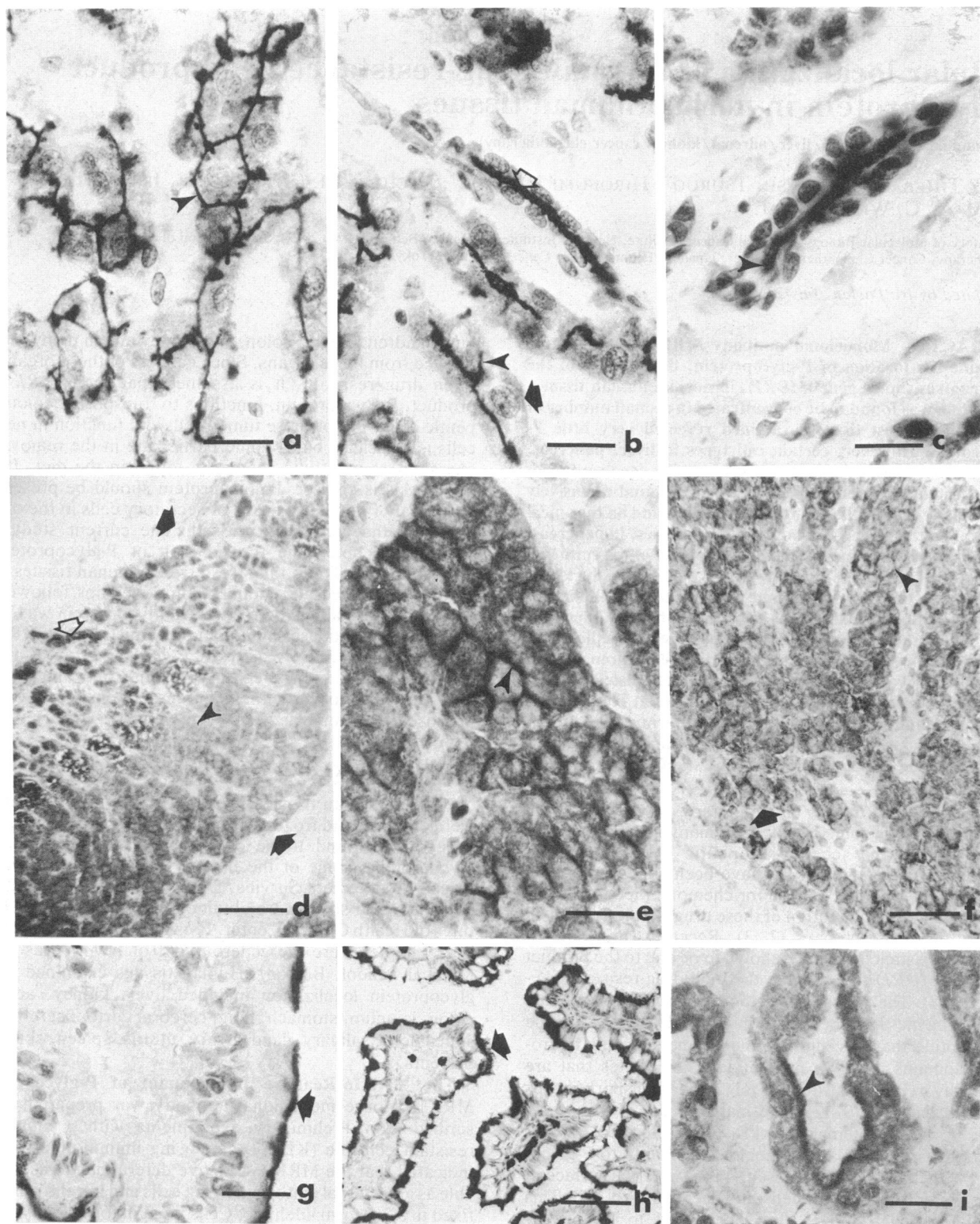


FIG. 1. Immunohistochemical peroxidase localization of P170 in normal human tissues. MRK16 monoclonal mouse antibody was used to localize P170 in normal human tissues as described in *Materials and Methods*. Major sites of localization were found in liver in biliary canaliculi (arrowhead) (a and b) and small biliary ductules (large open arrowhead) (b), in pancreas in small pancreatic ductules (arrowhead) (c), in adrenal cortex (d and e) and medulla (d and f), in colon on the apical surface of columnar epithelium (arrowhead) (g), in jejunum on the apical surface of columnar epithelium (arrowhead) (h), and in the kidney on the apical surface of proximal tubular epithelium (arrowhead) (i). The sinusoidal face of hepatocytes was not labeled (large dark arrowhead) (b). The large open arrowhead in d shows densely labeled cells in the glomerulosa; the upper large dark arrowhead in d marks the capsule of the adrenal; the lower large arrowhead marks the inner boundary of the adrenal medulla; the small arrowhead in d marks the reticularis. The arrowhead in e shows the surface labeling on cortical cells (glomerulosa); the large arrowhead in f shows labeling of the surface of medullary cells; the small arrowhead in f shows weaker labeling of cells in the adjacent reticularis of the cortex. Samples processed using a nonreactive mouse monoclonal antibody in place of MRK16 showed no labeling (not shown). (a, b, c, e, g, and i, $\times 600$; bars = $15\ \mu\text{m}$; d, $\times 95$; bar = $100\ \mu\text{m}$; f, $\times 240$; bar = $37\ \mu\text{m}$; h, $\times 335$; bar = $26\ \mu\text{m}$.)

ethanol showed continued reactivity with another anti-P-glycoprotein antibody, C219 (19) (Centocor, Malvern, PA).

Peroxidase Immunohistochemistry. Cryostat sections (6 μ m) of fresh-frozen normal human tissues were thaw-mounted on coverslips and allowed to air dry. In some cases, these coverslips were coated with Cell-Tak (Biopolymers, Farmington, CT). They were then fixed by immersion in 3.7% formaldehyde in phosphate-buffered saline (PBS) for 10 min at 23°C, washed in PBS, and then preincubated in 10% normal goat serum for 5 min prior to exposure to MRK16 antibody. The dilutions and conditions for the subsequent peroxidase immunohistochemical steps have been described (17).

RESULTS AND DISCUSSION

Immunohistochemical Localization. Using monoclonal antibody MRK16, high levels of P-glycoprotein were detected in liver, colon, jejunum, kidney, pancreatic ductules, and adrenal. In the liver, the protein was detected on the biliary canicular surface of hepatocytes and on the apical surface of small biliary ductules (Fig. 1 *a* and *b*). In the colon and jejunum, it was found on the apical surface of columnar epithelial cells (Fig. 1 *g* and *h*). In the kidney, P-glycoprotein was only detected on the apical surface of the epithelial cells of the proximal tubules (Fig. 1*i*). In addition, P-glycoprotein was found on the apical surface of small pancreatic ductules (Fig. 1*c*). In all these tissues, the protein was present in a highly polarized fashion.

However, in adrenal, the protein was not present in a polarized distribution. It was detected in both the adrenal cortex and the medulla (Fig. 1 *d-f*). In the cortex, the strongest reaction was in the glomerulosa and fasciculata, with a weaker reaction in the reticularis. The medulla showed uniform moderate reactivity on the surface of all medullary cells. Tissues that showed no detectable labeling included lung, stomach, salivary gland, cerebral cortex, cerebellum, spinal cord, ovary, uterus, skin, spleen, and placenta. Furthermore, many cell types from organs that were previously shown to express elevated MDR1 RNA levels (18), such as cells of the kidney glomerulus, were negative. In all cases, cells reactive with MRK16 failed to react with a control antibody (10 μ g/ml), confirming the specificity of the reaction. Nevertheless, we believe negative reactivity with MRK16 must be interpreted with caution because clearly positive results were only obtained with freshly frozen healthy tissues. It is clear that those tissues that showed high expression of the *MDR1* gene when RNA analyses were performed (18) also contained significant amounts of P-glycoprotein reactive with MRK16. The immunohistochemical localization results are summarized in Table 1, and a schematic drawing of these organ distributions is shown in Fig. 2.

The MRK16 Epitope Is on the External Surface of the Cell. Monoclonal antibody MRK16 recognizes P-glycoprotein in

human liver, but not in liver from mouse (which expresses *mdr* mRNA) or cynomolgous monkey (data not shown), indicating that it detects a human-specific P-glycoprotein epitope. Evidence that the human-specific epitope is on the external surface of cultured cells and includes the human peptide sequence was obtained by localizing MRK16 in mouse cells that had received the human *MDR1* gene in a DNA-mediated transfer experiment (20). As shown in Fig. 3, multidrug-resistant transfectant NIH 3T3 cells express human P-glycoprotein in a plasma membrane pattern. The same result was obtained when living cells were exposed to MRK16, confirming that the epitope is present on the external surface of the cell (result not shown) (17).

Significance of the Cellular Location of P-Glycoprotein. Most of the sites in tissues containing detectable amounts of P-glycoprotein are on the apical membranes of cells facing an excretory compartment. This localization suggests that the protein has a role as a pump for physiological metabolites and chemotherapeutic drugs. Only in the adrenal was the protein found to be diffusely distributed, suggesting that it might pump substances into the interstitial space instead of into a secretory system with a duct or into the lumen of the intestine. Many of the drugs affected by the multidrug-resistance phenotype are secreted in the bile and found in the gastrointestinal tract. One surprising finding of this study is the clear localization of P-glycoprotein to the apical surface of the columnar cells of the lower gastrointestinal tract. Since these cells represent a large fraction of the total P-glycoprotein-containing cells in the body, it seems likely that direct excretion of drugs into the lumen of the gastrointestinal tract may represent a major route of detoxification.

Therapeutic Possibilities. It has been suggested that if a protein responsible for multidrug resistance could be identified on the surface of cancer cells, it might be a good target for antibody-directed therapy (15). Using an immunotoxin composed of MRK16 coupled to *Pseudomonas* exotoxin (MRK16-PE), we have recently shown that multidrug-resistant cells expressing high levels of P-glycoprotein are readily killed by MRK16-PE, whereas cells not expressing the protein are not (21). The finding that normal tissues such as the liver and kidney expressed high levels of the mRNA encoding P-glycoprotein raised the possibility that MRK16-

Table 1. Localization of P-glycoprotein with MRK16

P-glycoprotein detected	P-glycoprotein not detected
Liver, biliary canicular surface of hepatocytes: apical surface of small biliary ductules	Stomach
Jejunum and colon: apical surface of columnar epithelial cells	Lung
Kidney: brush border of proximal tubules	Central nervous system (cerebral cortex, cerebellum, spinal cord)
Pancreas: apical surface of small ductules	Ovary and uterus
Adrenal: diffusely on the surface of cells in medulla and cortex	Spleen
	Skin
	Placenta
	All other cells of liver, jejunum, colon, kidney, and pancreas

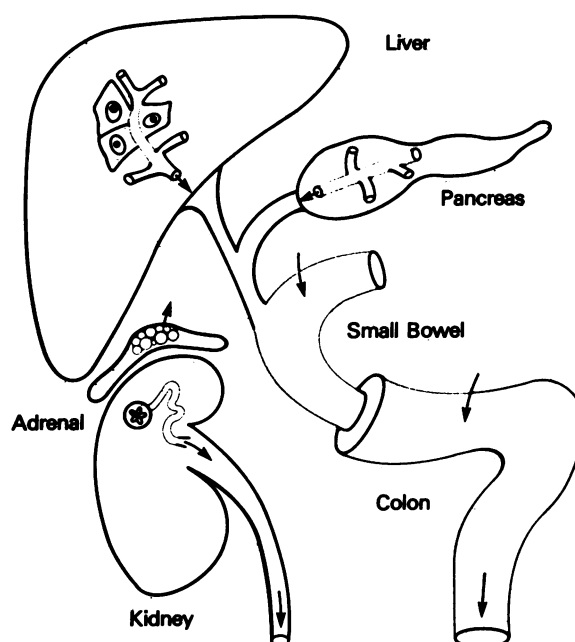


FIG. 2. Schematic drawing of the organ distributions of P-glycoprotein found using immunohistochemistry.

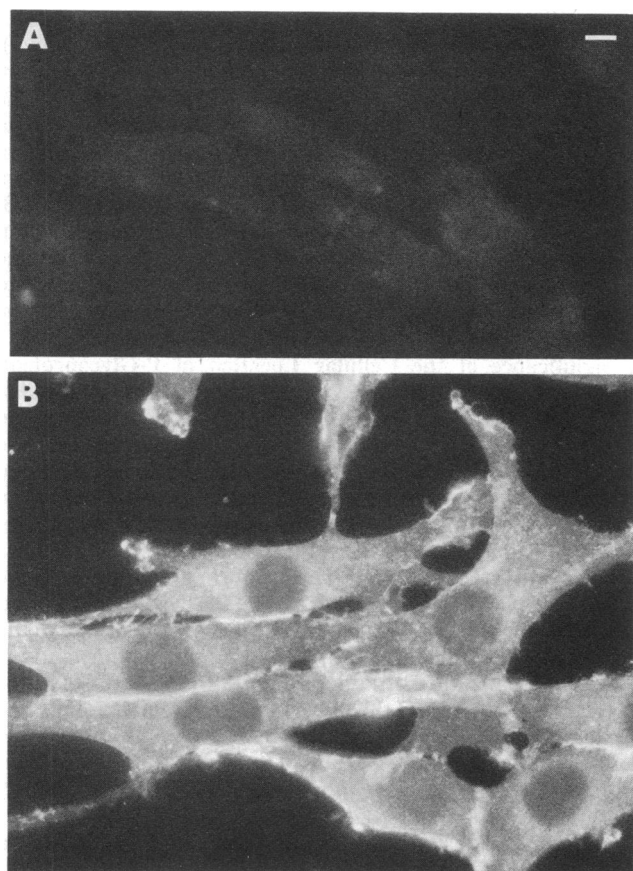


FIG. 3. Localization of P170 in mouse cells transfected with the human *MDR1* gene. NIH 3T3 mouse cultured fibroblasts were transfected with genomic human DNA derived from MDR KB cells and selected for MDR with colchicine. DNA from primary transfectant MDR NIH 3T3 cells was transferred to NIH 3T3 cells to yield secondary NIH 3T3 MDR transfectants (T_2 - C_1 cells), which express the human *MDR1* gene (20). Bright surface labeling of these mouse cells using MRK16 and immunofluorescence is shown in *B*, while MRK16 shows no labeling of nontransfected NIH 3T3 cells (*A*). These cells were primarily fixed with formaldehyde and subsequently exposed to MRK16 antibody in the presence of saponin. Similar results were obtained in experiments performed with living T_2 - C_1 cells; similar results were also obtained using clones from pHaMDR cells, cells that were transfected with cDNA derived from the *MDR1* gene (6) (results not shown). ($\times 425$; bar = 10 μ m.)

PE might destroy normal tissues and not be useful in killing drug-resistant cells. The current finding that in all tissues,

with the exception of the adrenal, P-glycoprotein is inaccessible to antibodies administered parenterally because of its location on the luminal surface of these organs suggests that immunotoxins or other antibody-directed therapies might yet be useful in the therapy of multidrug resistance.

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