Expression of the MRP2 Gene-Encoded Conjugate Export Pump in Human Kidney Proximal Tubules and in Renal Cell Carcinoma

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Abstract. Human kidney proximal tubule epithelia express the ATP-dependent export pump for anionic conjugates encoded by the MRP2 (cMRP/cMOAT) gene (symbol ABCC2). MRP2, the apical isoform of the multidrug resistance protein, is an integral membrane glycoprotein with a molecular mass of approximately 190 kD that was originally cloned from liver and localized to the canalicular (apical) membrane domain of hepatocytes. In this study, MRP2 was detected in human kidney cortex by reverse transcription-PCR followed by sequencing of a 826-bp cDNA fragment and by immunoblotting using two different antibodies. Human MRP2 was localized to the apical brush-border membrane domain of proximal tubules by double and triple immunofluorescence microscopy including laser scanning microscopy.

The expression of MRP2 in renal cell carcinoma was studied by reverse transcription-PCR and immunoblotting in samples from patients undergoing tumor-nephrectomy without prior chemotherapy. Clear-cell carcinomas, originating from the proximal tubule epithelium, expressed MRP2 in 95% (18 of 19) of cases. Immunofluorescence microscopy of MRP2 in clear-cell carcinoma showed a lack of a distinct apical-to-basolateral tumor cell polarity and an additional localization of MRP2 on intracellular membranes. MRP2, the first cloned ATP-dependent export pump for anionic conjugates detected in human kidney, may be involved in renal excretion of various anionic endogenous substances, xenobiotics, and cytotoxic drugs. This conjugate-transporting ATPase encoded by the MRP2 gene has a similar substrate specificity as the multidrug resistance protein MRP1, and may contribute to the multidrug resistance of renal clear-cell carcinomas.
(cMRP/cMOAT) gene has been localized to chromosome 10q24 (11). On the mRNA level, human MRP2 is expressed predominantly in the liver, in addition to kidney and small intestine (11,18–20).

In the present study, we extended our work on expression and localization of MRP2 in the rat kidney (7) to human kidney obtained from patients undergoing tumor-nephrectomy. Furthermore, MRP2 expression was studied in renal cell carcinomas, predominantly of the most frequent subtype, the clear-cell carcinoma (21–23). Primary and metastasizing renal cell carcinomas often exhibit an intrinsic resistance to different chemotherapeutic agents (23). Clinical trials with the cytostatic drug vinblastine, including dextrerapamil as a modulator of MDR1 P-glycoprotein-mediated multidrug resistance, showed no significant improvement of survival rates of patients with metastasizing renal cell carcinoma (24).

Materials and Methods

Human Tissue Samples

Renal cell carcinoma and corresponding nontumorous kidney cortex samples were obtained from 21 patients (mean age 63.5 yr; range, 47 to 89). None of the patients had received chemotherapy. After tumor-nephrectomy, the neoplastic and the normal kidney tissues were separated, snap-frozen in isopentane precooled in liquid nitrogen, and further processed for reverse transcription (RT)-PCR, immunoblotting, or immunofluorescence microscopy. The warm ischemia period of the tissues ranged from 15 to 30 min until freezing. Informed consent for tissue delivery was obtained from patients before tumor-nephrectomy.

Materials

The protease inhibitors phenylmethylsulfonyl fluoride, aprotinin, leupeptin, pepstatin, and protein standard mixture (M, 26,600 to 180,000) were purchased from Sigma Chemicals (Deisenhofen, Germany). RNase inhibitor (RNasin), StrataScriptM Moloney murine leukemia virus reverse transcriptase, Taq DNA polymerase, and β-actin primers were from Stratagene (Heidelberg, Germany). Other chemicals were of analytical grade and delivered by Merck (Darmstadt, Germany).

Antibodies

The EAG5 polyclonal antibody was raised in rabbits against a synthetic peptide containing the amino acid sequence EAGIENVNSTKF at the carboxy terminus of the human MRP2 protein, as described previously (9,25). The polyclonal antibody MLE was obtained by immunization of rabbits against the amino-terminal synthetic peptide of the human MRP2 (MLEKFCNSTFWNSSSLFD-SPEADLPCL) (9,18,25). The antibodies EAG5 and MLE did not cross-react with MRP1. The monoclonal mouse anti-CD26 antibody directed against human dipetidyl-peptidase IV was purchased from Dianova (Hamburg, Germany). Goat anti-rabbit secondary antibodies coupled to Texas red were from Dianova. Goat anti-mouse secondary antibodies coupled to FITC or cyanin 2-conjugate (Cy2) was from Biotrend (Cologne, Germany).

RNA Isolation, RT-PCR, and Subcloning

Total RNA was isolated from nontumorous kidney cortex and corresponding renal cell carcinomas by a guanidinium thiocyanate lysis procedure with subsequent purification by centrifugation on cesium chloride (26). To prevent amplification on genomic DNA, total RNA was treated, before reverse transcription, with 10 U of DNase I in 50 μl of digestion buffer (100 mM sodium acetate, pH 5.0, 5 mM MgSO4, and 40 U of the RNase inhibitor RNasin) at 37°C for 1 h. Then, total RNA (5 μg) was reverse-transcribed using an oligo(dT)18 primer for MRP1 as described (7), or a sequence-specific reverse primer for MRP2 (bases 4284 to 4267). The primer pairs used for PCR detection of the respective cDNA were based on the sequences from the GenBank/EMBL/DataBank with the accession nos. L05628 (MRP1; reference (8)) and X96395 (MRP2; reference (9)). For MRP1, the sense primer was 5’-ATCAAGACCCGTCTCAT-TGG-3’ (nucleotides 1183–1202), and the antisense primer was 5’-GAGCAAGGATGACTTGCAGG-3’ (nucleotides 1363–1344). For MRP2, the sense primer was 5’-CCACAGGGCGGATTG-3’ (nucleotides 3227–3243), and the antisense primer was 5’-AAGATTT-GAGAGGGCA-3’ (nucleotides 4052–4035). The commercial β-actin control primer pair was obtained from Stratagene. For each PCR, the cycling conditions were as follows: 94°C for 45 s; 60°C for 60 s; 72°C for 90 s (35 cycles). Amplified cDNA fragments were subcloned into the pCR2.1 vector (Invitrogen, Leek, The Netherlands). Positive clones were sequenced by the dideoxynucleotide chain termination method of Sanger using [α-35S]dATP and the sequencing kit from Pharmacia Biotech (Freiburg, Germany). Dried gels were exposed to Kodak BioMax MR-1 film obtained from Sigma (Deisenhofen, Germany).

Immunoblotting

Human kidney cortex and inner medulla or renal cell carcinoma tissue (about 1.0 g each) was homogenized during thawing in 10 ml of lysis buffer (10 mM KCl, 1.5 mM MgCl2, 10 mM Tris/HCl, pH 7.4) and 0.5% (wt/vol) sodium dodecyl sulfate supplemented with four protease inhibitors at the following final concentrations: 1 mM phenylmethylsulfonyl fluoride, 0.3 μM aprotinin, 1 μM pepstatin, and 1 μM leupeptin at 4°C (7). The resulting suspension was further processed for crude membrane preparation as recently described (7), and 25 or 50 μg of protein was loaded onto a 7.5% (wt/vol) sodium dodecyl sulfate-polyacrylamide gel, without boiling, and subjected to electrophoresis (27). After electrotransfer onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany), the blots were blocked with Tris-buffered saline containing 0.05% Tween 20 and 10% (wt/vol) low-fat dry milk (Glücksklee, Frankfurt, Germany) for 1 h at room temperature and probed overnight with the polyclonal MRP2 antibodies EAG5 and MLE at a dilution of 1:20,000, respectively. Antibody binding was visualized with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Bio-Rad, Munich, Germany) diluted 1:1000, followed by enhanced chemiluminescence detection with exposure on HyperfilmTM-MP (Amersham-Buchler, Braunschweig, Germany). A semiquantitative score was used for the signal intensity of the 190-kD band corresponding to MRP2 after application of 50 μg of protein: + ++ + + + + + ; strong; + + + positive; + + weak; – no reactivity for MRP2 using the EAG5 antibody. Canalicul membrane from human liver (9,18) served as an MRP2-rich positive control.
**Immunofluorescence Microscopy**

Samples from frozen kidney cortex and corresponding renal cell carcinoma were used for single and double-label immunofluorescence microscopy. Unfixed tissue sections (4 to 5 μm thick) were prepared with a cryotome (Leica, FrigoCut 2800E; Nussloch, Germany) and air-dried for 2 to 4 h at room temperature. Before the incubation with the antibodies, the sections were fixed in 100% acetone precooled at −20°C for 10 min. For double- or triple-label immunofluorescence microscopy, the primary (rabbit EAG5, 1:100; mouse anti-CD26, 1:100 or 1:200; mouse anti-calbindin-D, 1:50 or 1:100; sheep anti-Tamm-Horsfall glycoprotein, 1:80 to 1:120) as well as the secondary antibodies (goat anti-mouse or anti-rabbit, or donkey anti-sheep) were applied simultaneously. Incubation with the primary and secondary antibodies was for 30 to 40 min each at room temperature. Unbound antibodies were removed by several washes with phosphate-buffered saline after each incubation step. As negative controls for the specific reactivity of the EAG5 and MLE antibodies, both preimmune sera were used. After a final wash with distilled water, the air-dried sections were mounted with Elvanol (CTI, Idstein/Taunus, Germany).

As a further control, peptide competition with the EAG5 antibody was performed. For preabsorption of the EAG5 antibody, the synthetic peptide used to create this antibody (amino acids 1534–1545 of MRP2) was solubilized at concentrations of 100 and 10 μM in the antibody solution and allowed to bind for 30 min at room temperature before its application to the tissue sections. Fluorescence imaging micrographs were taken with a Zeiss-Axiophot microscope (Carl Zeiss, Jena, Germany). FITC or Cy2 and Texas red were excited at 495 nm and 560 nm, respectively. Photographs were taken on Kodak T-MAX 400 film. Confocal images in double or triple fluorescence were obtained with a Carl Zeiss LSM 510 ultraviolet laser scanning microscope. For our photographs, we used an argon ion laser (488 nm) and a HeNe laser (543 nm) with the corresponding barrier filters in the double track mode of the instrument (i.e., each scan line was alternatively illuminated only with one wavelength to avoid bleed-through of the fluorescence dyes) for double fluorescence. For simultaneous triple fluorescence, we used an additional argon ion laser (364 nm) for the excitation of amino-methylcoumarin acetate.

**Histopathology**

Tissue samples for diagnostic histopathology were formaldehyde-fixed, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. All tumor specimens were reviewed independently by a reference pathologist (S.S.). Tumor progression was estimated according to the postoperative tumor staging classification (28). Grading of tumor tissue was recorded as follows: well differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3).

**Table 1. MRP2 immunoreactivity in patients with renal-cell carcinoma**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Stage (TNM)b</th>
<th>Grade (G)c</th>
<th>Histopathology</th>
<th>MRP2 Expressiond</th>
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<tr>
<td></td>
<td>RCC Kidney</td>
<td>Cortex</td>
<td>Cortex 12</td>
<td>12</td>
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<tr>
<td>1</td>
<td>pT2N0M1</td>
<td>G1</td>
<td>Clear-cell</td>
<td>++++</td>
</tr>
<tr>
<td>2</td>
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<td>G1</td>
<td>Clear-cell</td>
<td>+++</td>
</tr>
<tr>
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<td>G1</td>
<td>Clear-cell</td>
<td>+++</td>
</tr>
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<td>G1</td>
<td>Clear-cell</td>
<td>+++</td>
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<tr>
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<td>G2</td>
<td>Clear-cell</td>
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</tr>
<tr>
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<td>Clear-cell</td>
<td>++</td>
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<tr>
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<td>Clear-cell</td>
<td>++</td>
</tr>
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<td>G2</td>
<td>Clear-cell</td>
<td>++</td>
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<td>G2</td>
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<td>++</td>
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<td>G2</td>
<td>Clear-cell</td>
<td>++</td>
</tr>
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<td>++</td>
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<td>Clear-cell</td>
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<td>Clear-cell</td>
<td>++</td>
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<td>G2</td>
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<td>++</td>
</tr>
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<td>G3</td>
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</tr>
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<td>G3</td>
<td>Clear-cell</td>
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<td>G3</td>
<td>Clear-cell</td>
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</tr>
<tr>
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<td>++</td>
</tr>
<tr>
<td>19</td>
<td>pT4N0M0</td>
<td>G3</td>
<td>Clear-cell</td>
<td>−</td>
</tr>
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</table>

a Patients underwent tumor surgery without preoperative chemotherapy or irradiation treatment. The mean age of all patients undergoing tumor-nephrectomy was 63.5 yr (range, 47 to 89).

b pTNM, postoperative tumor staging (28).

c Pathohistologic tumor grading.

d Immunoreactivity for MRP2: +++, strong; ++, positive; +, weak; −, negative.

e No kidney tissue available because of nephron-sparing surgery.
Detection of MRP2 mRNA in Human Kidney and in Renal Cell Carcinomas

The expression of MRP2 (symbol ABCC2) and MRP1 (symbol ABCC1) genes in kidney cortex and renal cell carcinoma from tumor-nephrectomized patients (cortex 12 and 20; renal cell carcinoma 12 and 20) was analyzed by reverse transcription and PCR amplification of cDNA fragments generated from isolated total RNA (Figure 1). The amplification products showed the expected size of 826 bp for MRP2 and 181 bp for MRP1 (Figure 1). As an internal control, the β-actin 661-bp cDNA fragment demonstrated the integrity of the isolated total RNA from the tissue specimens (Figure 1). Amplification products corresponding to MRP2 and MRP1 cDNA fragments were obtained from normal kidney cortex (cortex 12 and 20). The MRP2 and MRP1 cDNA fragments were further identified by subcloning and sequencing of 826 nucleotides for MRP2 and of 181 nucleotides for MRP1. Both the MRP2 and MRP1 cDNA fragments showed a nucleotide sequence identity of 99.5% compared with the cloned sequences from human liver (MRP2; references (9) and (18)) and H69AR cells (MRP1; reference (8)). We have analyzed the mRNA from a total of six well preserved renal clear-cell carcinomas and consistently detected the amplification product of the MRP2 cDNA. MRP2 mRNA was below detectability in the two chromophilic renal cell carcinomas studied, but we did obtain an amplification product for MRP1 mRNA (Figure 1).

Immunoblot Analysis of MRP2 in Human Kidney and Renal Cell Carcinomas

Immunoblots on crude membrane preparations from kidney cortex and from the corresponding renal cell carcinoma samples (Table 1) were probed with polyclonal antibodies directed against human MRP2. The antibody EAG5 indicated a strong expression of the 190-kD glycoprotein in all kidney cortex samples (Figures 2 and 3; Table 1). In addition, the MLE antibody, directed against the amino terminus of MRP2, detected the protein at a molecular mass of approximately 190 kD in kidney cortex (Figures 2 and 3c).

Eighteen of 19 clear-cell carcinoma samples (95%) were positive for MRP2 expression using the antibody EAG5. This result was confirmed by the MLE antibody. Clear-cell carcinomas with G1 grading semiquantitatively showed a more intense immunoreactivity of MRP2 than the less differentiated tumors graded G3 (Table 1).

Figure 2. Immunoblot showing MRP2 expression in crude membranes from human kidney cortex and a corresponding clear-cell renal carcinoma (RCC 4) (patient 4; Table 1). Detection of MRP2 with protein A-Sepharose-purified antibodies MLE and EAG5 shows the protein of approximately 190 kD.

Figure 3. Immunoblot of MRP2 expression in crude membrane fractions from human kidney cortex, inner medulla, and renal cell carcinoma from patients with clear-cell carcinoma (patients 3 and 12). MRP2 was detected with antibodies EAG5 and MLE as a protein of approximately 190 kD in all kidney cortex samples and in clear-cell carcinoma (RCC 12). No reactivity for MRP2 was seen in kidney medulla regions (A) and in the two chromophilic renal cell carcinomas studied (RCC 20 in B and C).
Figure 4. Single-label (a) and double-label (b through g) immunofluorescence microscopy of MRP2 and dipeptidyl-peptidase IV in human adult kidney cortex. Tissue sections (from samples 1 and 5; Table 1) show fluorescent signals with the antibody EAG5 (EAG) directed against human MRP2 (a, b, d, and f). As a marker protein for the apical (luminal) membrane domain of renal proximal tubules, the monoclonal antibody anti-CD26 directed against human DPPIV (DPP) was applied (29). Proximal tubule epithelia near the glomerulus (G) showed staining at the luminal brush-border membrane with the EAG5 (filled triangles in a, b, d, and f) and anti-DPPIV antibodies (filled triangles in c, e, and g). Both antibodies did not react with tubular segments distal from the proximal tubules (open triangles). Poor immunoreactivity in some areas is a result of the period of warm ischemia during surgery and prior to freezing of the tissue (filled circles in d and e). Occasionally, autofluorescent particles can be detected in some tissue slices (arrowheads in a). Bars: 50 μm in a through e; 100 μm in f and g.
Localization of MRP2 and Dipeptidyl-Peptidase IV in Human Kidney and Renal Cell Carcinomas

MRP2 was localized in human kidney cortex by immunofluorescence microscopy. The EAG5 antibody indicated a fluorescence signal only in proximal tubule epithelia. The reaction product was predominantly localized in the luminal (apical) brush-border membrane domain (Figure 4, a, b, d, and f). The nephron segments following the proximal tubule, i.e., thin limbs of Henle’s loop, thick ascending limb of Henle’s loop including the macula densa, distal collecting tubule, and the cortical and medullary collecting ducts, were negative for MRP2 (Figure 4). Dipeptidyl-peptidase IV (CD 26; EC 3.4.14.5) (DPPIV), a marker for the apical membrane domain (29), clearly colocalized with MRP2 on proximal tubule brush-border membranes (Figure 4, b through g).

MRP2 localization in different specimens of clear-cell carcinomas indicated a loss of the regular apical-to-basolateral polarity observed in normal proximal tubule epithelial cells (Figure 5, a and c). An apparent localization of MRP2 on intracellular membranes was detected in many of the clear-cell carcinoma cells. MRP2-positive cells also expressed DPPIV as shown by double-label immunofluorescence (some are indicated by arrowheads in Figure 5). In a high number of clear-cells, reaction products with DPPIV were localized to intracellular membranes. A localization of MRP2 together with DPPIV to the plasma membrane of the tumor cells was observed only occasionally (e.g., small arrows in Figure 5d). In many clear-cell carcinomas, a dissociation of MRP2 and DPPIV expression was observed, particularly with cells that were DPPIV-positive but MRP2-negative (Figure 5).

The immunofluorescence specificity of the EAG5 antibody was tested by two different experiments. Either the secondary antibody was used without prior incubation with primary EAG5 antibody, or the EAG5 antibody was preabsorbed with the synthetic peptide that had been used to raise this antibody. In both experiments, the normal staining of the apical membrane domain of proximal tubules (Figure 4, a, b, and d) did not appear (Figures 6, b and c). This proved that the secondary antibody used did not create an unspecific staining pattern and that the specific antibody reaction could be blocked by preincubation with the proper peptide. The specificity of this reaction was further confirmed by the fact that preincubation with EAG5 peptide did not influence the reac-

Figure 5. Double-label immunofluorescence microscopy of MRP2 and DPPIV in a compact-growing clear-cell renal carcinoma. Tissue sections (from tumor sample 15; Table 1) were stained with the antibodies EAG5 (EAG; a and c) and anti-DPPIV (DPP; b and d). Although only a subpopulation of the carcinoma cells expressed MRP2 (some corresponding cells in Panels a and b and Panels c and d that show colocalization with both antibodies are indicated by arrowheads in a and c), most of the clear cells were positive for DPPIV (b and d). Small arrow in Panel a points to cellular extensions of MRP2-positive cells. Bar, 50 μm.
activity of the DPPIV antibody (Figure 6d) as shown by double-label immunofluorescence (Figure 6, c and d).

To further support the finding that MRP2 is restricted to proximal tubules, we performed double- and triple-label experiments using antibodies to MRP2 in combination with antibodies against Tamm-Horsfall glycoprotein, marking the thick ascending limb and the early distal convoluted tubule (30,31). In addition, we used antibodies to calbindin, reacting with the distal convoluted and connecting tubule (32,33). All three antibodies stained different nephron segments in the kidney cortex (Figure 7, a and b, and Figure 8, a through d). In medullary regions, however, only antibodies to Tamm-Horsfall glycoprotein reacted positively (Figure 7, c and d). These results demonstrate that human MRP2 is detectable only in the apical membrane domain of proximal tubules.

**Discussion**

In the present study, we demonstrate the expression of MRP2 in the apical membrane of human kidney proximal tubule epithelia. Moreover, we describe the expression of MRP2 in clear-cell renal carcinoma. Renal cell carcinoma is the most common malignancy of human adult kidney and accounts for 2 to 3% of all cancers (23). Approximately 80% of all renal cell carcinomas belong to the clear-cell subtype originating from proximal tubular epithelia (21–23). The intrinsic multidrug resistance phenotype of renal cell carcinomas is still a major obstacle for chemotherapy of patients with primary and metastasizing adult kidney cancer (24,34).

MRP2 was detected in all nontumorous, human kidney cortex samples (Table 1), and its localization was confined to the apical membrane domain of proximal tubule epithelia (Figures 4 and 8). MRP2, also termed the hepatocyte canalicular multidrug resistance protein, cMRP (9,35), or canalicular multispecific organic anion transporter, cMOAT (10,11), has a broad substrate specificity for endogenous and xenobiotic amphiphilic anions similar to the substrate specificity established for the MRP1 transporter (12–17,36). This substrate specificity may account at least for some of the hitherto described active transport processes for organic anions from renal proximal tubules into the luminal space (1–4). Conjugation or complex formation of endogenous compounds and cytotoxic agents with glutathione and other anionic groups is often a prerequisite for transport by MRP1 and MRP2 (12–18,36). Transfection studies with MRP1 cDNA in HeLa cells (reviewed in reference (16)) and work by Koike et al. (20) suggest that not only MRP1 but also MRP2 expression confers multidrug resistance to cancer cell lines. In the present study, we observed that MRP2 is expressed in 95% of the renal cell carcinomas with a clear-cell pathohistology (Table 1). The localization of MRP2 in clear-cells significantly differs from nontumorous kidney proximal tubule epithelia because of the predominant intracellular localization of this transport protein. It is presently not known...
to what extent the MRP2 in intracellular membranes can be sorted to the plasma membrane of tumor cells when exposed to cytostatic drugs. However, recent studies by Cui et al. (37) indicate that MRP2 expression in cells confers multidrug resistance. Our semiquantitative analyses shown in Table 1 suggest that MRP2 expression might also correlate with the differentiation status of renal clear-cell carcinomas. MRP2 may be below detectability in dedifferentiated clear-cell tumors with G3 grading. Further investigations are required to clarify whether MRP2 expression is negatively correlated with the dedifferentiation of renal clear-cell carcinoma or with chromosomal aberrations during tumor progression.

In conclusion, we propose that MRP2 under physiologic conditions functions in renal elimination of endogenous and xenobiotic amphiphilic anions from blood into urine. MRP2 has the capacity to confer multidrug resistance (37) and, when sorted to the plasma membrane, may contribute to the resistance of renal clear-cell carcinoma to a wide variety of cytotoxic chemotherapeutic agents. Future studies should define whether MRP2 is the only ATP-dependent export pump secreting amphiphilic organic anions into the lumen of kidney proximal tubules or whether additional members of the MRP family of transporters, which were identified recently (19), contribute to this process.

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**Figure 7.** Double-label immunofluorescence of frozen sections of adult human kidney cortex (Co; a and b) and medulla (Me; c and d) after reaction with EAG5 for MRP2 (E; a and c) and Tamm-Horsfall (T; b and d) antibodies. In cortex areas, the antibodies clearly reacted with different segments of the nephron (a and b). Some structures including the glomeruli (G) were negative for both antibodies (arrowheads in a and b). In medullary regions (Me; c and d), the EAG5 (E) antibody to MRP2 was negative (c), while the antibody to Tamm-Horsfall protein (T) strongly reacted with various tubular segments (d). Bar (shown in b), 100 μm; same magnification for all panels.
Figure 8. Confocal laser scanning micrographs of frozen sections of human kidney after double (a through c) and triple (d) immunofluorescence labeling. Positive reactions with the EAG5 antibody to MRP2 (E; a and c) are seen on proximal tubules (some are marked with filled triangles in a), while other tubular segments are unstained (open triangles in a). After application of calbindin antibodies (C; b and c), positive reactions are seen on distal convoluted and connecting tubules (open triangles in b), leaving other tubular segments unstained (closed triangles in b). The merged image of a and b (c) reveals that the staining patterns of the EAG5 antibody for MRP2 (E, green fluorescence) and the calbindin antibody (C, red fluorescence) do not overlap. Triple-label fluorescence, shown in Panel d, with antibodies to calbindin (C, blue fluorescence), Tamm-Horsfall protein (T, red/orange fluorescence), and MRP2 (E, green fluorescence), demonstrates that the three different antigens are located at different cortical tubular segments of the nephron. G, glomerulus. Bars (shown in b and d), 100 μm; same magnification for panels a through c.
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References


