Differentiation dependent expression of P-glycoprotein in the normal and neoplastic human kidney

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Abstract. Adult renal cell carcinoma (RCC) is clinically resistant to chemotherapy. However, in nephroblastoma (NBL) chemotherapy has increased survival dramatically. We studied the P-glycoprotein (P-gp) expression of 18 RCC and 9 NBL as well as 1 benign renal adenoma and fetal renal tissue using three different monoclonal antibodies (MRK-16, C-219, JSB-1). P-gp was found positive with all three antibodies in 12/18 RCC, while only 2 tumors were completely negative. Staining varied with respect to intensity and number of positive cells [5%–90%]. Intense staining was seen at the apical side of malignant tubules in well differentiated parts of RCC and in tubular structures of the benign renal adenoma. Poorly differentiated parts of the tumors showed less staining. In NBL blastemal parts were negative. In 4/8 specimens showing focal epithelial differentiation, however, the luminal side of more differentiated tubular structures did stain, strongly resembling P-gp staining in the developing fetal human kidney. These results indicate that P-gp expression in normal (fetal) human kidney as well as in benign and malignant tumors derived from this organ depends on the degree of differentiation of tubules, which may have implications for chemotherapy sensitivity in both malignant tumors.

Key words: P-glycoprotein, immunohistochemistry, differentiation, renal cell cancer, nephroblastoma, fetal kidney

Introduction

Renal cell carcinoma (RCC) is one of the tumor types most resistant to chemotherapy. It accounts for 1%–2% of all cases of cancer and originates in proximal tubules [1]. Approximately one-third of patients have disseminated disease at the time of diagnosis and approximately 50% of those operated upon with curative intent will develop metastases at a later date. The results of numerous clinical trials investigating cytostatic drugs are uniformly poor [2]. In contrast, in childhood nephroblastoma, an embryonal tumor originating in metanephric blastema, antineoplastic agents, especially actinomycin-D, vincristine and doxorubicin, have proven to be very effective [3]. Most likely, differences in responsiveness to chemotherapy between these tumors originating in a single organ can largely be explained by the effectiveness of cytostatic drugs at the tumor cell level. In this light recent progress in elucidating the mechanism of multidrug resistance (MDR) to antineoplastic agents seems relevant.

In vitro studies have revealed that MDR goes along with cross resistance to a group of cytostatic drugs, including those that are effective in NBL. Such drugs are mainly natural products, but their structures and mechanisms of action are unrelated. The MDR phenomenon appears to be related to a decreased intracellular accumulation and changes in intracellular distribution of these drugs [4–6]. A major feature of MDR cells is the overexpression of the 170–180 kd membrane protein, P-glycoprotein [7], which is thought to be responsible for an energy-dependent outward transport of the drugs. Among normal human organs with excretory functions this protein has been detected at the luminal side of proximal tubules in the kidney [8, 9], suggesting an excretory function of toxic substances in renal physiology. Elevated mdr1 mRNA levels in RCC and other tumors originating in P-gp-expressing tissues have been reported [10–14]. The level of expression seemed to correlate with grade, and enhancement of in vitro doxorubicin sensitivity by quinidine was observed in tumors showing high mdr1 mRNA expression [14]. An immunohistochemical method which allows assessment of expression at the cellular level was developed and described [9]. To circumvent the potential lack of specificity of monoclonal antibodies, a panel of three anti-P-gp monoclonal antibodies (Moabs) was used. Staining characteristics of these Moabs after acetone fixation in normal human tissues and in a limited number of malignant tumors, including a limited number of
malignant tumors, including a limited number of RCCs, were also described [9]. In the present study we compared acetone fixation with histological fixation methods used by other groups for P-gp immunohistochemistry [15, 16]. Moreover, we studied expression at the cellular level and distribution in RCC and NBL. Results were compared with the staining pattern in the developing human kidney and in a benign renal adenoma. The potential role of P-gp in chemotherapy sensitivity of these tumors is discussed.

Patients and methods

Patients

The primary tumors of 16 patients with non-metastasized RCC and 1 with metastasized RCC (17) were included in this study. None of the patients had received any chemotherapy prior to operation. Their mean age was 58.8 ± 10.7 years (range 33–73) and mean tumor size 8.7 ± 4.2 cm. The primary tumor of patient 18 was obtained at autopsy. Patient 19 had a benign renal adenoma. A second group included primary tumor samples of 9 children with NBL. Their mean age was 2.4 ± 1.2 years and mean tumor size 12.9 ± 3.1 cm. These patients all received preoperative chemotherapy with vincristine and actinomycin-D. A subpleural metastasis was obtained from patient 20. All tumors were carefully reviewed by one of us (PvdV) with respect to histologic type and grade. Patient data are presented in Table 2. Fetal kidney tissue (13–20 weeks) was also collected for this study.

Cell lines

The human melanoma cell line BRO and a multidrug-resistant subline (BRO/pFRmrd1.6clone1.1), transfected with the mdr-1 gene [17], were obtained from Dr. P. Borst (Department of Molecular Biology, Netherlands Cancer Institute, Amsterdam). The human lung squamous cell carcinoma-derived doxorubicin-resistant SW-1573 series was developed and characterized in the department of Human Genetics at the Free University [18]. The cells were used as positive and negative controls and to test the effect of different fixatives. They were cultured under laboratory conditions as described earlier [4].

Antibodies

Three MoAbs directed against different epitopes of the P-glycoprotein molecule were used (C-219, MRK-16 and JSB-1). C-219 [15], which is of the IgG2b isotype, was kindly provided by Centocor (Tongeren, Belgium). The working dilution was 1:100, resulting in a final concentration of 10 µg/ml. MRK-16 [16, 19], kindly provided by Dr. Tsuruo, Tokyo, Japan, is an IgG2a antibody which recognizes an epitope on the extra-cellular side of P-glycoprotein. It was used in a dilution of 1:400. The final concentration used was 4 µg/ml. JSB-1, an IgG1 antibody, was raised in our laboratory [20]. JSB-1 ascites was used in a 1:100 dilution. Since hexapeptide analysis in Dr. Ling’s laboratory (Toronto, Canada) did not reveal any specific binding (personal communication), JSB-1 is believed to recognize a conformational epitope of the P-gp molecule, most probably at the inner side of the plasma membrane, since some permeabilisation is required for the reaction with JSB-1 to occur.

Fixatives

Using well documented tumor cell lines (BRO; BRO/pFRmrd1.6clone1.1; SW-1573; SW-1573/2R160) and some P-gp-expressing normal tissues (liver, kidney, adrenal, colon) different fixatives were compared. JSB-1 and C-219 were tested after Bouin fixation; 3-step fixation [15]: formaldehyde 0.46% (5 min 0 °C)/ethanol 95% (5 min −20 °C)/acetone (10 min −20 °C); formaldehyde 3.7% (10 min 23 °C) and acetone (10 min 0 °C). MRK-16 was tested on unfixed cells and on cells fixed in glutaraldehyde 0.1% (30 min 23 °C), ethanol 95%, formaldehyde 3.7% (10 min 23 °C) and acetone (10 min 0 °C).

Tissue handling and immunocytochemistry

A specimen of each tumor was snap frozen in liquid nitrogen and stored until use. Cryostat sections (5 µm) or cytopsin preparations (tumor cell lines) were fixed in cold acetone (10 min 0 °C) and air dried before use. For immunohistochemical studies an avidine-biotine complex (ABC) immunoperoxidase method (Histostain-SP kit, Zymed laboratories Inc., San Francisco, California) was used. The slides were developed with aminoethyl carbazole (AEC), counterstained with haematoxiline and mounted with aquamount. Phosphate-buffered saline (pH 7.4) was used for antibody dilution and for all washes. Cytopsin preparations of sensitive human squamous cell lung carcinoma cells (SW-1573) and their resistant sublines (SW-1573/2R160 [29] and SW 1573-2R500 [18]) were used as negative and positive controls, respectively. Negative control experiments also included the use of an unrelated (mouse anti-immunoglobulin) antibody in all experiments. Staining results were judged independently by three of us (PvdV, CJLMM and CvK). Cells or tissue sections were scored for each moab on number of positive cells and whenever possible for membranous or intracytoplasmatic staining at the cellular level and for preferential localisations of the positive cells in tissue specimen.
Table 1. Reactivity of JSB-1, C-219 and MRK-16 with human tumor cell lines using different histological fixation methods.

<table>
<thead>
<tr>
<th>Fixations</th>
<th>BRO</th>
<th>BRO-mdr</th>
<th>SW-1573</th>
<th>SW-1573/2R160</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>JSB-1</td>
<td>C-219</td>
<td>MRK-16</td>
<td>JSB-1</td>
</tr>
<tr>
<td>Acetone (10 min -20 °C)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+ + + 70</td>
</tr>
<tr>
<td>Formaldehyde/ethanol/cold acetone</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+ + + 70</td>
</tr>
<tr>
<td>Bouin</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>++</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
</tbody>
</table>

- = negative.
+ = weak staining (no membranous accentuation).
++ = clear positive staining (no membranous accentuation), accentuation in Golgi region.
+++ = membranous accentuation.
++++ = intense membranous accentuation.
1 = percentage of positive cells.

BRO = human melanoma cell line; BRO-mdr 1.1 = human melanoma cell line transfected with the mdr 1 gene; SW-1573 = human squamous cell lung carcinoma cell line (sensitive parental cells); SW-1573/2R160 = resistant subline of the human squamous cell lung cancer cell line SW-1573.

Results

Fixation experiments

The results of the fixation experiments for JSB-1, C-219 and MRK-16 are shown in Table 1. In cytospin preparations of cells of the different tumor cell lines the 3-step fixation also used by Chan [15] showed somewhat better results than acetone fixation for JSB-1 and C-219 with respect to intensity of staining and morphology of the cells. However, in cryostat sections of four P-gp-expressing normal human tissues (liver, colon, kidney, adrenal) this fixation revealed a weak signal. Bouin- and formaldehyde (3.7%) fixations were not feasible for P-gp immunohistochemistry with JSB-1 and C-219, showing only weak, non-membrane accentuated staining in BRO/pFRmdr1.6clone.1 and SW-1573/2R160. While formaldehyde, staining in the sensitive cell lines (BRO and SW-1573) was also observed. The signal after formaldehyde fixation for MRK-16 was comparable to that observed with acetone; however weak non-membrane accentuated staining was also seen with the negative control antibody. The reactivity of MRK-16 with its epitope was lost when ethanol (95%) and glutaraldehyde (0.1%) were used. Heterogeneity of staining intensity was observed in both mdr lines (BRO/pFRmdr1.6clone.1 and SW-1573/2R160) with all three moabs using the different fixations. This observation was clearest with acetone, formaldehyde/ethanol/cold acetone and formaldehyde (MRK-16) fixations but was also seen with the weaker fixatives (Table 1). C-219 showed a more pronounced heterogeneity than did JSB-1 and MRK-16.

Renal cell carcinoma

The results of P-gp immunohistochemistry in 18 primary RCC specimens are shown in Table 2. Sixteen of eighteen tumors showed positivity with at least two of the three moabs. However, as can be seen in the table, differences in staining intensity and number of positive cells (heterogeneity) were observed between the moabs. Clear apical staining at the luminal side of malignant tubules could be observed in well differentiated parts of RCC, mimicking the staining pattern in normal human proximal tubules. This was observed in 2 specimens (# 2, 13) (Fig. 1A, B). In less differentiated parts, with the ‘classical’ RCC appearance (large clear cytoplasm, central nucleus with 1-3 nuclei) 5%-90% P-gp positive cells were detected (Fig. 1A, B, C, D, F; Table 2). The number of positive cells varied between the different Moabs in each tumor (Table 2). Three tumors (# 1, 16, 17) were completely negative (Fig. 1E). Two tumors (# 15, 18) were negative with C-219, but showed faint positivity in 5%-10% of the cells with JSB-1 and MRK-16. The two sarcomatoid variants in this study (# 17, 18) showed no and limited staining, respectively. In one tumor (# 7) clear staining of the vascular endothelium, most pronounced with C-219 but also seen with the other Moabs, was observed (Fig. 1F). Positive cells were not found to be preferentially localised in, for instance, invading or dividing cells. In general, positive staining of tumor cells in RCC is faint and not clearly membranous in 10/15 positive cases. Membranous staining was most clearly observed with C-219 (# 10, 12, 20, Table 2).

Patient 18, showing very rapidly progressive lung metastasis, was treated with doxorubicin and cyclo-
Table 2. Patient data and P-gp reactivity in kidney tumors.

<table>
<thead>
<tr>
<th>Age</th>
<th>TNM class</th>
<th>Size</th>
<th>Diagnosis</th>
<th>P-gp</th>
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<td></td>
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<td>JSB-1</td>
</tr>
<tr>
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<td>49</td>
<td>T3a N1 M0</td>
<td>10.5</td>
<td>RCC</td>
</tr>
<tr>
<td>2.</td>
<td>65</td>
<td>T3a N1 M0</td>
<td>13.5</td>
<td>RCC</td>
</tr>
<tr>
<td>3.</td>
<td>53</td>
<td>T3b N0 M0</td>
<td>7</td>
<td>RCC</td>
</tr>
<tr>
<td>4.</td>
<td>47</td>
<td>T3b N0 M0</td>
<td>7</td>
<td>RCC</td>
</tr>
<tr>
<td>5.</td>
<td>71</td>
<td>T3b N0 M0</td>
<td>6</td>
<td>RCC</td>
</tr>
<tr>
<td>6.</td>
<td>49</td>
<td>T3b N0 M0</td>
<td>6</td>
<td>RCC</td>
</tr>
<tr>
<td>7.</td>
<td>72</td>
<td>T2 N0 M0</td>
<td>15</td>
<td>RCC</td>
</tr>
<tr>
<td>8.</td>
<td>59</td>
<td>T3b N1 M0</td>
<td>4</td>
<td>RCC</td>
</tr>
<tr>
<td>9.</td>
<td>33</td>
<td>T3a N1 M0</td>
<td>18</td>
<td>RCC</td>
</tr>
<tr>
<td>10.</td>
<td>49</td>
<td>T2 N0 M0</td>
<td>8</td>
<td>RCC</td>
</tr>
<tr>
<td>11.</td>
<td>64</td>
<td>T2 N0 M0</td>
<td>6</td>
<td>RCC</td>
</tr>
<tr>
<td>12.</td>
<td>66</td>
<td>T2 N1 M0</td>
<td>9</td>
<td>RCC</td>
</tr>
<tr>
<td>13.</td>
<td>63</td>
<td>T2 N0 M0</td>
<td>5</td>
<td>RCC</td>
</tr>
<tr>
<td>14.</td>
<td>60</td>
<td>T2 N0 M0</td>
<td>5</td>
<td>RCC</td>
</tr>
<tr>
<td>15.</td>
<td>67</td>
<td>T2 N0 M0</td>
<td>7</td>
<td>RCC</td>
</tr>
<tr>
<td>16.</td>
<td>73</td>
<td>T2 N0 M0</td>
<td>6</td>
<td>RCC</td>
</tr>
<tr>
<td>17.</td>
<td>73</td>
<td>T2 N0 M0</td>
<td>6</td>
<td>RCC</td>
</tr>
<tr>
<td>18.</td>
<td>45</td>
<td>T3b N3 M1</td>
<td>15</td>
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<td>19.</td>
<td>63</td>
<td>T1</td>
<td>1</td>
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<tr>
<td>20.</td>
<td>5</td>
<td>I</td>
<td>14</td>
<td>NBL</td>
</tr>
<tr>
<td>20A.</td>
<td>6</td>
<td>IV</td>
<td>NBL(m)</td>
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<tr>
<td>21.</td>
<td>1</td>
<td>I</td>
<td>10</td>
<td>NBL</td>
</tr>
<tr>
<td>22.</td>
<td>3</td>
<td>I</td>
<td>12</td>
<td>NBL</td>
</tr>
<tr>
<td>23.</td>
<td>2</td>
<td>I</td>
<td>15</td>
<td>NBL</td>
</tr>
<tr>
<td>24.</td>
<td>3</td>
<td>I</td>
<td>18</td>
<td>NBL</td>
</tr>
<tr>
<td>25.</td>
<td>2</td>
<td>I</td>
<td>13.5</td>
<td>NBL</td>
</tr>
<tr>
<td>26.</td>
<td>1</td>
<td>I</td>
<td>7</td>
<td>NBL</td>
</tr>
<tr>
<td>27.</td>
<td>3</td>
<td>I</td>
<td>14</td>
<td>NBL</td>
</tr>
<tr>
<td>28.</td>
<td>2</td>
<td>I</td>
<td>13</td>
<td>NBL</td>
</tr>
</tbody>
</table>

phosphamide because of the unknown origin of the primary tumor at that time. The patient failed to respond and died soon after the first cycle. At autopsy she was diagnosed as having a sarcomatoid RCC. P-gp immunohistochemistry of the primary tumor showed a very faint staining with JSB-1 and MRK-16 (in 10% and 5% of the cells, respectively), but not with C-219. The results with the cell line (HiB2) derived from pericardial effusion withdrawn from this patient were described earlier [21]. In this cell line some staining with JSB-1 and MRK-16, but not with C-219, was also observed. Mdr1-mRNA in this cell line (RNA-se protection assay, Dr. F. Baas, The Netherlands Cancer Institute, Amsterdam), was just detectable (not shown).

**Nephroblastoma**

The blastemal parts of all 9 NBLs were negative for P-gp (Fig. 1G; Table 2). In four tumors (# 20, 24, 25, 27) showing focal epithelial differentiation, however, staining was detected at the luminal side of a few primitive tubules (Fig. 1G, H). In one tumor (# 22) which showed rhabdomyomatous differentiation, staining of striated muscle fibers was seen with C-219 (Fig. II). Staining with JSB-1 and MRK-16 was negative. All patients received preoperative chemotherapy with vincristine and actinomycin-D, followed by radical nephrectomy and consolidation chemotherapy with the same drugs. One patient (# 20) developed lung metas-

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**Fig. 1.** Reactivity of RCC, NBL and fetal kidney with anti-P-gp antibodies. IA: RCC (# 13) with C-219, showing clear apical staining of well differentiated tumor cells (indicated by arrowheads 1), showing a cribiform growth pattern with false lumina. Less differentiated cells show less accentuated staining (indicated by arrowheads 2), × 200. IB: Detail of Fig. 1A. Note the strong apical staining of well differentiated RCC cells. Faint intracytoplasmic staining of the cells represents endogenous biotin activity also seen with the negative control antibodies, × 630. IC: RCC (# 12) with MRK-16, heterogeneous staining of tumor cells, × 160. ID: RCC (# 14) with JSB-1, Faint apically accentuated staining of tumor cells, exhibiting classical RCC appearance, × 400. IE: Sarcomatous RCC (# 17) with C-219. No staining detectable in sarcomatous (undifferentiated) variants of RCC in this study, × 630. IF: RCC (# 7) with C-219. Preeminent staining of tumor vessels as compared to the intensity of staining of tumor cells in this tumor, × 400. IG: NBL (# 20) with C-219. Blastemal part of NBL (indicated by arrow 1), not showing any staining for P-gp. In parts of this tumor showing epithelial differentiation (indicated by arrow 2) apical staining of tubular structures is seen, × 160. IH: NBL (# 24) with JSB-1. Detailed picture of primitive tubules clearly showing apical staining, × 400. II: NBL (# 22) with C-219. Striated muscle fibre staining of an NBL showing rhabdomyomatous differentiation, × 630. 1J: Fetal kidney (15 weeks) with C-219. Clear apical staining of tubules (arrowhead 1) and Bowman's capsule (arrowhead 2).
tases during consolidation chemotherapy and died of progressive disease. No increase in P-glycoprotein expression could be detected in the metastases, which consisted only of blastemal cells, as compared to the blastemal part of the primary tumor (Fig. 1G).

Fetal kidney

In fetal kidney material clear positive staining for P-gp was observed in tubules and in Bowman's capsule at different timepoints (13–20 weeks) during fetal kidney formation (Fig. 1J). The number of P-gp-expressing tubules increases with age and maturity of nephrons. Glomeruli and metanephric blastema were negative (Fig. 1J).

Discussion

P-gp overexpression has been shown to play an important role in multidrug resistance in a number of tumor cell lines [22]. Thus, its expression in human tumor specimens may indicate the presence of the MDR phenotype in patients. Increased mdr-1 mRNA and P-gp expression has been found in several human cancers [9, 10] as well as in some normal tissues [8, 9, 16, 23]. Some recent reports have shown frequent overexpression of mdr-1 mRNA in RCC [9–11]. The present study compares P-gp expression in RCC, NBL and in normal (fetal) kidney. The two tumor types represent chemo-resistant and chemo-sensitive cancer types, respectively. Because of the contrasting response to chemotherapy of the two tumors, originating in a single organ, we compared by immunohistochemistry, the degree of homogeneity of P-gp expression at the cellular level, an aspect which cannot be determined with bulk techniques such as northern (mRNA) and western (protein) blotting.

From the fixation procedures we tested in this study, cold acetone fixation appeared to be the most useful for the Moabs used in tissue sections because it can be used for all three Moabs and allows clear positive results in normal and malignant human tissues. The 3-step fixation (formaldehyde, ethanol, acetone) with JSB-1 and C-219 showed a weaker staining in tissue sections from P-gp-expressing organs. This is probably caused by the superior penetration of formaldehyde (0.46%), which is known to destroy the epitopes for JSB-1 and C-219 in tissue sections [15, 20]. MRK-16 binds to unfixed as well as to formaldehyde-fixed cells [8, 24]. Indeed, clear positive results were obtained with this Moab using formaldehyde, while somewhat weaker staining and deteriorated morphology were observed in unfixed cells. However, no satisfactory negative controls (unrelated antibody, sensitive cell line) could be obtained using formaldehyde.

Contrary to the suggestion that intracytoplasmic staining with JSB-1 in cells of low resistance might represent the presence of P-gp [21, 25], it has now become clear that JSB-1 may show some cross-reactivity of as yet unknown significance in tumor cell lines not expressing P-gp [29]. A faint background staining with JSB-1 was also observed in tissue slides. C-219 is known to recognize a 200 kd protein in striated- and heart muscle [9, 16] which does not interfere with interpretation of the slides. Indeed, in one case we observed clear C-219 muscle fibre staining in a NBL with rhabdomyomatous differentiation (Fig. 11). The cross-reaction pattern of MRK-16 resembles that of JSB-1 in producing a very faint background signal in some sensitive cell lines and in human tissue specimens which was also seen in normal guinea pig tissues (results not shown). MRK-16 only recognizes human P-gp [16].

In tissue sections C-219 seems to be the most specific Moab, producing clearer positive results without background staining (see Fig. 1; Tables 1, 2). Its sensitivity in cases of very low expression, at least compared to that of JSB-1 and MRK-16, is not yet clear. C-219 as well as JSB-1 have been shown to be able to detect MDR cells with low levels of resistance (4–6 times that of sensitive cells) [15, 26]. MRK-16 seems at least as sensitive as C-219 and JSB-1.

In well differentiated parts of RCC with a tendency to tubule formation, apical staining, mimicking the staining pattern in normal proximal tubules, was observed (Fig. 1A, B). The clear cell variant, which is the most common histologic type in RCC, showed faint heterogeneous positivity (Fig. 1C, D, F), less pronounced than that of normal tubular cells or 'tubular' differentiated tumor cells (Fig. 1A, B). This observation is in agreement with the higher mdr1-RNA levels described in well differentiated or grade 1–2 tumors as compared to poorly differentiated (grade 3–4) RCC [13]. In a benign renal adenoma (# 19) exhibiting proliferation of well differentiated tubules, clear apical staining was again observed. Although histological grading to some extent contributes to prognosis, surgical and pathologic T status are more important prognostic factors in patients with RCC [31]. However, patients with rapid progression tend to suffer from poorly differentiated or sarcomatoid subtypes [30]. Patient 18 showed rapidly progressive advanced disease. The tumor was classified post mortem as a sarcomatoid RCC. The very faint staining (with JSB-1 and MRK-16, but not with C-219) in the primary tumor (5%–10% of the cells) and in the cell line derived from tumor cells of the patient [21] as well as the barely detectable mdr1-mRNA message in this cell line (not shown) do not suggest a major role for P-gp in the observed chemoresistance in this particular patient.

No increased positivity could be observed in vaso-inivating or stroma infiltrating cells in this study. Studies focusing more intensively on this issue would be of particular interest because the levels of mdr1-mRNA expression in metastatic lesions were recently reported to be increased over those in the primary tumors of two patients [13]. It has been suggested that
P-gp expression in vascular endothelium of capillaries at blood-tissue barrier sites (brain, testis) plays a role in the limited ingress of cytostatic agents into these organs [16,23]. In this respect, the observation of P-gp positivity in capillary blood vessels of one RCC (#7) may point to a mechanism of clinical importance, justifying further investigation.

Not a single positive cell could be detected in blastemal parts of NBL specimens (Fig. 1G) nor was positive staining seen in a subpleural pulmonary metastatic lesion (#20) consisting only of undifferentiated blastemal cells. The lesion had developed during consolidation chemotherapy and thus it seems unlikely that clinical chemotherapy resistance in this patient was mediated by P-gp. In four tumors in which focal epithelial (tubular) differentiation was observed, clear apical staining was detected (Fig. 1G, H), which strongly resembled P-gp expression in the developing human kidney (Fig. 1J). Since an apparently more favourable outcome is observed among patients with tumors consisting predominantly of tubular and glomerular structures and in patients with tumors showing higher degrees of differentiation in general [27,28], we may conclude that P-gp expression should not be considered as a marker for chemotherapy resistance in this particular disease. In any case it does not appear to be a marker for unfavourable prognosis. On the contrary, as an indicator of grade, the number of P-gp-expressing tubules is more likely to correlate with a good prognosis.

We have shown that immunohistochemistry using three different Moabs is an easily applicable and sensitive technique to study cellular P-gp expression. Nevertheless, caution is warranted with the interpretation of cases showing very faint staining or staining with only one or two Moabs of the panel.

Our results strongly indicate that P-gp expression in the developing human kidney as well as in benign and malignant human kidney tumors correlates with the level of differentiation of the tubules. The question of whether this presumed differentiation-dependent P-gp expression does play a role in clinical chemotherapy resistance, especially in advanced RCC, remains to be answered. Low- or undifferentiated tumor cells (heterogeneous expression) comprise a substantial part of practically all RCCs [31]. Very low levels of P-gp expression at the cellular level in these undifferentiated cells, resulting in an additional 2–3-fold resistance, may not be detected with this immunohistochemistry method. In view of the narrow therapeutic window of anticancer drugs, a clinical significance for such levels cannot be excluded. However, small but significant in vitro enhancement of doxorubicin cytotoxicity by quinidine was observed only in those RCCs expressing high mdr1-mRNA levels [13]. In vitro resistance to vinblastine seemed to correlate better with mdr1-RNA levels in RCC [12]. Therefore, more definite answers must await the outcome of studies focusing on functional parameters of MDR, including the accumulation and intracellular distribution of drugs in freshly obtained patient tumor cells.

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References


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St. Gallen, Switzerland 26–29 February 1992

*International Consensus Conference with review of all aspects of adjuvant chemo-, endocrine-, and radiotherapy as well as surgical techniques and psycho-social problems in major studies world-wide.*

*The official language of the Conference will be English.*

*There will be scientific posters and also an industrial exhibition.*

Deadline for abstracts: October 31, 1991

*The Conference will be held in the OLMA-Messe, Kongresshalle 2, St. Gallen, Switzerland*

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