

Nephroblastoma

Multidrug-Resistance P-Glycoprotein Expression in Tumor Cells and Intratumoral Capillary Endothelial Cells

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Abstract

The development of chemoresistance in a variety of cancers seems related to overexpression of the P-glycoprotein (P-gp) drug pump. Nephroblastoma, the most common malignant renal tumor of childhood, usually is responsive to treatment, and prognosis is favorable in most cases. However, the disease in a subset of patients is refractory to treatment, and the disease follows an aggressive course.

To study P-gp expression in this tumor and its correlation with outcome, tumor samples from 93 patients were examined by immunohistochemical analysis. P-gp expression was determined separately in both tumor cells and intratumoral capillary endothelium. The likelihood ratio test, the Kaplan-Meier method, and the log-rank test were used to evaluate its association with clinical course, grade, stage, and administration of preoperative chemotherapy.

The results for the majority of nephroblastomas were variably positive; in 43 (46%) of them, newly formed capillary endothelial cells also stained positive. While no association of P-gp expression in tumor cells with clinical course, stage, and grade could be demonstrated, positivity in endothelial cells correlated significantly with unfavorable outcome, suggesting that chemoresistance depended on an active blood-tumor barrier. Previous chemotherapy induced P-gp overexpression in tumor cells.

Human P-glycoprotein (P-gp, MDR1 [multidrug resistance] gene product) is an energy-dependent plasma membrane glycoprotein that can extrude a variety of structurally diverse, toxic xenobiotic compounds from cells. Because P-gp also can bind multiple antitublastic drugs, such as dactinomycin, vinca alkaloids, anthracyclines, epipodophyllotoxins, and paclitaxel,¹ it is considered one of the key molecules involved in multidrug resistance in cancer.

P-gp originally was identified in drug-resistant cells, but is now known to be normally expressed in several tissues,² and an excretory, protective, or hormone-handling role has been suggested. Tumors arising from these tissues often inherently express the multidrug transporter and are generally intrinsically refractory to anticancer agents of natural origin.^{3,4}

Nephroblastoma (Wilms tumor [WT]), the most common malignant renal tumor of childhood, is a highly chemosensitive tumor.^{5,6} However, in 20% of cases, treatment cannot control the progression of disease, and death occurs in 50% of patients who experience relapse. Since WT originates from kidney, which normally expresses P-gp,^{2,7,8} drug resistance mediated by P-gp might represent an important obstacle to successful treatment in these aggressive cases.

The aim of the present study was to investigate the correlation between the degree of P-gp expression and resistance to chemotherapy, evaluated in terms of risk of both relapse and death. In addition, we studied whether previous chemotherapy might induce P-gp overexpression in tumors of pretreated patients.

We performed immunohistochemical analysis on paraffin-embedded samples from 93 WTs, 72 of which had been pretreated and 21 had not. We used 4 different monoclonal anti-P-gp antibodies that already had proved effective in

specifically determining the external domain, even in the presence of very low glycoprotein levels. All methods were in accordance with the consensus recommendations by Beck et al.⁹

Materials and Methods

Patients

We retrospectively obtained 93 records for children affected by nephroblastoma with at least 12-month follow-up and available tissue from the files of “Bambino Gesù” Children’s Hospital, Rome, Italy. Data regarding sex, age, treatment, and clinical course were recorded. Criteria for staging and grading were according to the last protocol (93-01) of the Société Internationale d’Oncologie Pédiatrique (SIOP).¹⁰

Records for patients who received preoperative chemotherapy were separated from those who underwent surgery directly. Clinical course was subdivided into overall survival (OS) and disease-free survival (DFS) categories, from date of diagnosis, as based on the initial diagnostic imaging, according to SIOP guidelines. In addition, 2- and 5-year survival and 2- and 5-year DFS were considered for patients with appropriate length of follow-up.

Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded tissue sections were dewaxed in xylene, rehydrated in an ethanol dilution series, and then incubated for 20 minutes in 3% hydrogen peroxide to block endogenous peroxidase. After washes in tris(hydroxymethyl)aminomethane-buffered saline, the sections were treated with 20% normal bovine serum and incubated at room temperature for 60 minutes with 4 different mouse monoclonal antibodies against P-gp: F4 (NeoMarkers, Union City, CA), MC57 (IgG2a kappa; final concentration, 12.5 µL/mL),¹¹ MM10.12 (IgG2a kappa; pretreatment with 5% pepsin at 37°C for 10 minutes; final concentration, 15 µL/mL),¹² and MM4.17 (IgG2a kappa; final concentration, 20 µL/mL) (MC57, MM10.12, and MM4.17 were gifts of M.C.).¹³ After washing, the slides were reincubated with biotinylated antimouse IgG (DAKO, Copenhagen, Denmark) at room temperature for 30 minutes, followed by further incubation with an avidin and biotinylated horseradish peroxidase complex (DAKO) at room temperature for 30 minutes. Chromogenic development was obtained using 3,3'-diaminobenzidine tetrahydrochloride with 0.03% hydrogen peroxidase (DAKO). Finally, sections were counterstained with hematoxylin, cleared, and mounted. Sections without primary antibodies served as the negative control, while normal kidney adjacent to tumor served as the positive control.

Two pathologists (F.D.C. and R.B.) independently performed light microscopic evaluation of stained sections without knowledge of patient characteristics. As P-gp reaction was not distributed uniformly, they randomly selected 15 tumor fields and obtained a final mean value for each tumor.

Tumor cells and intratumoral capillary endothelium expression was evaluated separately using the following scale: 0, negative (0%-5% positive cells); 1+, weak (6%-15%); 2+, moderate (16%-30%); 3+, strong (31%-50%); and 4+, very strong (>50%). Proximal tubule epithelium positivity was arbitrarily considered as 3+. The staining degree of each antibody was recorded separately, and the 4 results were finally summarized to yield a single score (range, 0-16+) for both tumor cells and intratumoral capillary endothelium.

Statistical Methods

For statistical analyses, we divided tumor cells and intratumoral capillary endothelium P-gp expression values into 3 groups: negative (0), weak/moderate (1-8+), strong/very strong (9-16+). Stages also were divided into 3 groups: low (I, II/negative lymph nodes), high (II/positive lymph nodes, III, IV), and bilateral (V).

The likelihood ratio test was used to evaluate the association between P-gp expression and grade, stage, and administration of preoperative chemotherapy.

The Kaplan-Meier method was applied to assess the potential relationship of OS, 2-year survival, 5-year survival, relapse-free survival (RFS), 2-year RFS, and 5-year RFS with P-gp expression. The log-rank test was used to compare the Kaplan-Meier curves.

In each analysis, the elaboration was performed separately for tumor cells and intratumoral capillary endothelium.

Results

Clinicopathologic Features

Of 93 children, 50 were boys and 43 were girls. The mean age at diagnosis was 43.8 months (range, 2-183 months). In all cases, the diagnosis of nephroblastoma and the clinical stage were established initially by means of diagnostic imaging (abdominal ultrasound and computed tomography of chest and abdomen with intravenous contrast).

Patients were treated according to SIOP protocols (SIOP-6, SIOP-9, and SIOP-93-01) that recommend preoperative chemotherapy and vary little in terms of therapeutic agents and duration of treatment.¹⁰ Preoperative chemotherapy was administered to 72 patients: 58 with localized tumor received a 2-drug regimen (dactinomycin

Table 1
Degree of Immunohistochemical P-Glycoprotein Positivity in Tumor Cells According to Pathologic Stage and Grade*

Wilms Tumor (n = 93; 72/21)	Negative (n = 9; 5/4)	Weak (n = 25; 14/11)	Moderate (n = 34; 28/6)	Strong (n = 21; 21/0)	Very Strong (n = 4; 4/0)
Pathologic stage					
I (n = 44)	2/3	5/5	15/2	11/0	1/0
II- (n = 18)	1/0	4/3	4/1	5/0	—
II+ (n = 7)	1/0	1/1	2/0	1/0	1/0
III (n = 10)	0/1	1/2	3/3	—	—
IV (n = 3)	—	—	1/0	—	2/0
V (n = 11)	1/0	3/0	3/0	4/0	—
Risk grade					
Low (n = 7)	1/1	2/2	1/0	—	—
Intermediate (n = 72)	3/3	11/7	24/5	17/0	2/0
High (n = 14)	1/0	1/2	3/1	4/0	2/0

* Total number of patients (n) is subdivided into patients given/not given preoperative chemotherapy. Stage II-, negative lymph nodes; stage II+, positive lymph nodes.

and vincristine), and 14 with metastatic disease at diagnosis received a 3-drug regimen (dactinomycin, vincristine, and doxorubicin/epirubicin). Previously untreated patients included the remaining 21 children who had undergone surgery first for the following reasons: 6 were younger than 6 months of age, 7 had been referred after previous surgery, 4 had a doubtful radiologic diagnosis, and 4 had an acute abdomen. Total or partial nephrectomy was performed in all patients; intraoperative rupture occurred in 2 cases.

Pathologic stage distribution was as follows: stage I, 44 cases; stage II/negative lymph nodes, 18 cases; stage II/positive lymph nodes, 7 cases; stage III, 10 cases; stage IV, 3 cases; and stage V, 11 cases. Microscopically, intermediate risk histologic features were predominant (72/93 [77%]), while low and high risks represented 8% (7/93) and 15% (14/93), respectively.

Postoperative treatment (dactinomycin, vincristine, doxorubicin, epirubicin, carboplatin, ifosfamide, etoposide, and radiotherapy) was based on surgical and histopathologic staging.¹⁰ Chemotherapy, radiotherapy, and metastasectomy whenever feasible were performed in 25 patients who experienced relapse, with incomplete excision and/or tumor rupture occurring in 5 cases. At the end of the study, 15 patients had died of disease, 1 was alive with disease, 5 were completing therapy, and the remaining 72 had completed treatment and were in complete remission.

Immunohistochemical Findings

In the majority of cases (80%), the 2 observers concurred in grading the percentage of P-gp positivity; in the remaining cases, there were minor variations in grading, and the final value was achieved after mutual agreement. When P-gp was expressed, it was detected by all 4 antibodies; however, the intensity of staining generally was greater with MM4.17 and MC57 and weaker with the F4 clone.

The results for 84 nephroblastomas (90%) were variably positive. Among them, 59 (70%) showed weak or moderate tumor cell P-gp expression (42 pretreated and 17 untreated tumors). The remaining 25 (30%) showed strong or very strong positivity, and all were pretreated tumors. Tumor cell P-gp expression according to stage and grade is shown in **Table 1**.

Distribution of positivity reflected fetal and normal kidney reaction patterns^{7,8}: areas of tubular structures generally stained positive, while glomeruloid structures did not. Staining reaction was evident on the cellular membrane, as well as in the cytoplasm in immature epithelial elements, while it was observed prevalently on the luminal border in well-differentiated tubular structures **Image 1A**. A weak or moderate cytoplasmic reaction of the blastematos component often was observed **Image 1B** and **Image 1C** and was more intense in treated tumors. The mesenchymal component was negative, except for the rhabdomyoblastic elements of treated tumors, which were positive (Image 1C).

Endothelial cells of intratumoral capillaries expressed P-gp in 43 nephroblastomas (46%) **Table 2**: weak or moderate positivity was observed in 33 cases, which included 25 pretreated and 8 untreated tumors. Ten pretreated tumors showed intense P-gp stain (Images 1B and 1C). Only 1 pretreated tumor showed moderate positivity in newly formed capillary endothelium but negativity in neoplastic cells **Image 1D**.

Statistical Analysis

The mean length of follow-up was 71 months for OS (range, 6-185 months; median, 64 months), 83 months for 2-year survival (77 patients included), and 94 months for 5-year survival (59 patients included); 59 months for RFS (range, 3-185 months; median, 45 months), 69 months for 2-year RFS (77 patients included), and 75 months for 5-year RFS (61 patients included).

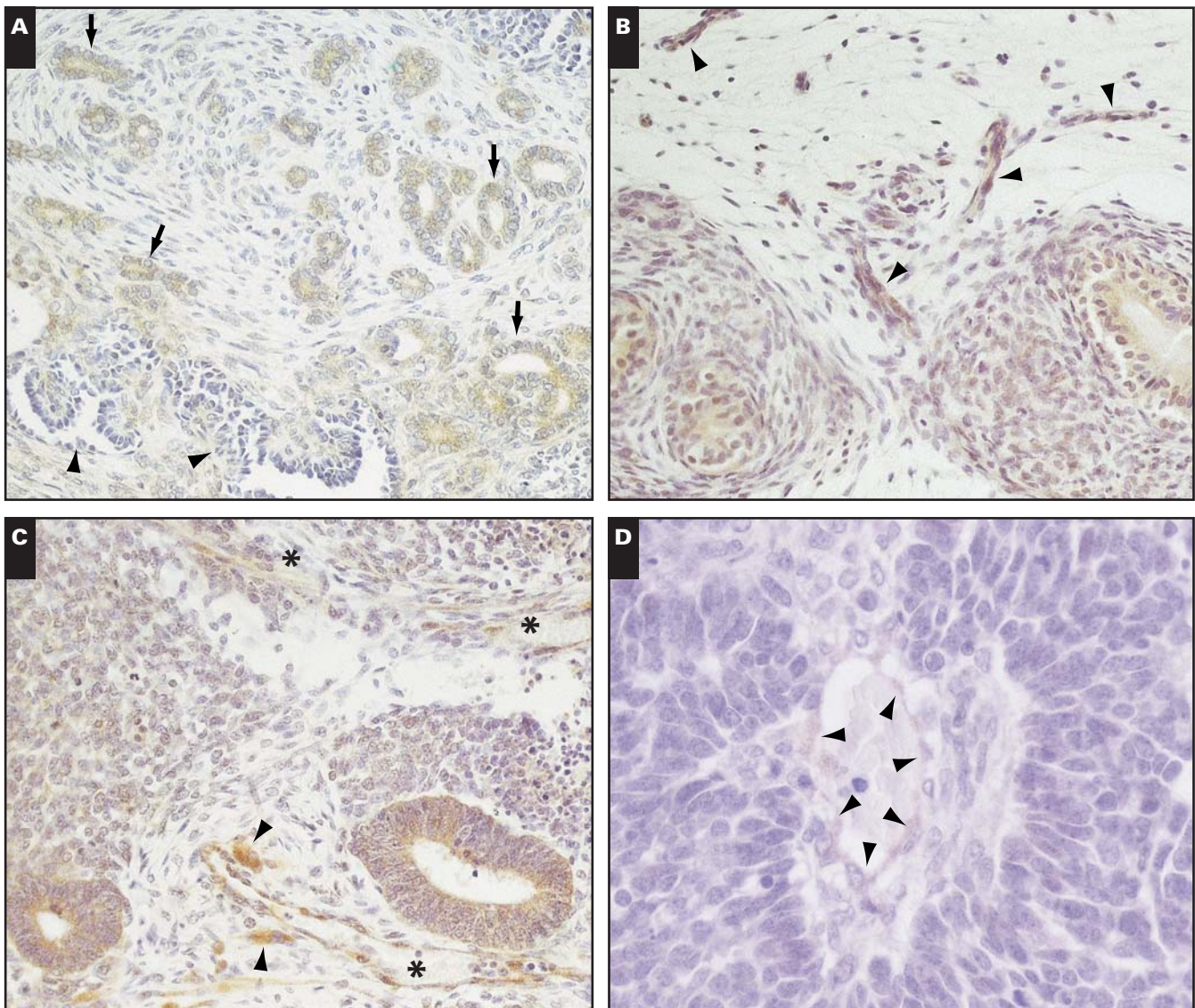


Image 1 Immunohistochemical findings. **A**, Wilms tumor (WT) scored 2+. The epithelial component was positive in tubular structures. Luminal border in well-differentiated tubules showed a stronger staining (arrows). Glomeruloid structures (arrowheads) and mesenchymal component did not stain (MC57 antibody, original magnification $\times 20$). **B**, WT scored 3+. Both the epithelial and blastematos components were positive. Intratumoral capillaries demonstrated strong positivity (arrowheads) (MC57 antibody, original magnification $\times 20$). **C**, WT scored 4+. The tumor showed an intense positivity, especially in tubular structures. Scattered rhabdomyoblastic elements were positive (arrowheads). Endothelium of small intratumoral vessels strongly expressed P-gp (asterisks) (MM4.17, original magnification $\times 20$). **D**, WT scored 0. The blastematos tumor did not stain; however, endothelial cells of newly formed capillaries showed moderate positivity (arrowheads) (MC57 antibody, original magnification $\times 40$). See the "Materials and Methods" section for a description of the scoring system.

Intratumoral capillary endothelium P-gp expression was correlated with an unfavorable outcome. The percentage of P-gp–stained endothelial cells increased with tumor aggressiveness, with only 1 relapse and 1 death in 50 patients with negative endothelium, 12 relapses and 8 deaths in 33 with moderately positive endothelium, and 10 relapses and 6 deaths in 10 with strongly positive endothelium. The OS and 5-year survival Kaplan-Meier analyses stratified by P-gp

expression showed statistically significant differences for intratumoral capillary endothelium (OS, $P < .00001$; 5-year survival, $P = .0016$) but not for tumor cells (OS, $P = .15$; 5-year survival, $P = .021$). The same analysis for 2-year survival was not applicable because of insufficient death events in the strata.

The RFS, 2-year RFS, and 5-year RFS Kaplan-Meier analyses stratified by P-gp expression showed statistically

significant differences for intratumoral capillary endothelium (RFS, 2-year RFS, and 5-year RFS, $P < .00001$) **Figure 1** but not for tumor cells (RFS, $P = .052$; 2-year RFS, $P = .07$; 5-year RFS, $P = .059$).

Increasing P-gp expression showed no significant association with grade (tumor cells, $P = .033$; intratumoral capillary endothelium, $P = .035$), and no association with stage could be demonstrated. Previous chemotherapy correlated significantly with increased P-gp expression in tumor cells ($P = .001$) but not in intratumoral capillary endothelium ($P = .84$).

Discussion

Few previous studies have investigated whether P-gp was expressed more strongly by WT displaying aggressive as opposed to favorable behavior, but given the small series studied, no conclusive results could be obtained.¹⁴⁻²⁰ The present study included 93 nephroblastomas. We chose immunohistochemical analysis because it is a sensitive, specific, reliable, and rapid method, and, because the morphologic features of the tissue are preserved, it permits localization of the examined antigen. The concordant staining of 4 antibodies that recognize 4 different epitopes is a strong argument for the actual presence of P-gp.⁹

Our results revealed that the majority of nephroblastomas showed a variable degree of P-gp positivity in tumor cells and that administration of chemotherapy enhanced its expression. Given the broad positivity of tumors, a clear relationship between the presence of P-gp and the chemoresistant phenotype of unfavorable WTs could not be demonstrated, even when different degrees of expression were considered. The evidence that P-gp was also expressed frequently in responsive tumors suggested that this multidrug transporter did not affect the critical intracellular concentration of administered drugs. Some hypotheses could be formulated to explain this discrepancy: (1) a competitive mechanism, in which the efflux system is engaged in pumping off xenobiotics released by neoplastic cells; (2) a drug-affinity selection, in which the multidrug transporter more specifically binds one type of drug, allowing the other(s) to efficiently reach the cellular target; and (3) a functional defect, in which the P-gp molecule synthesized by the tumor harbors conformational modifications in the intracellular part that is important in signal transduction.^{21,22} In addition, the involvement of other well-known drug transporters, such as the multidrug resistance-associated protein and the lung resistance-related protein,^{1,4} were not studied in WT and, thus, could not be ruled out. Concerning P-gp overexpression in pretreated WTs, a finding observed also by other authors,¹⁴ it might be attributed to a direct stimulating effect

Table 2
Degree of Immunohistochemical P-Glycoprotein Positivity in Intratumoral Capillary Endothelial Cells According to Pathologic Stage and Grade*

Wilms Tumor (n = 93; 72/21)	Negative (n = 50; 37/13)	Weak or Moderate (n = 33; 25/8)	Strong or Very Strong (n = 10; 10/0)
Pathologic stage			
I (n = 44)	21/7	10/3	3/0
II- (n = 18)	8/1	5/3	1/0
II+ (n = 7)	2/1	2/0	2/0
III (n = 10)	2/4	2/2	—
IV (n = 3)	—	—	3/0
V (n = 11)	4/0	6/0	1/0
Risk grade			
Low (n = 7)	3/2	1/1	—
Intermediate (n = 72)	31/11	19/4	7/0
High (n = 14)	3/0	5/3	3/0

* Total number of patients (n) is subdivided into patients given/not given preoperative chemotherapy. Stage II-, negative lymph nodes; stage II+, positive lymph nodes.

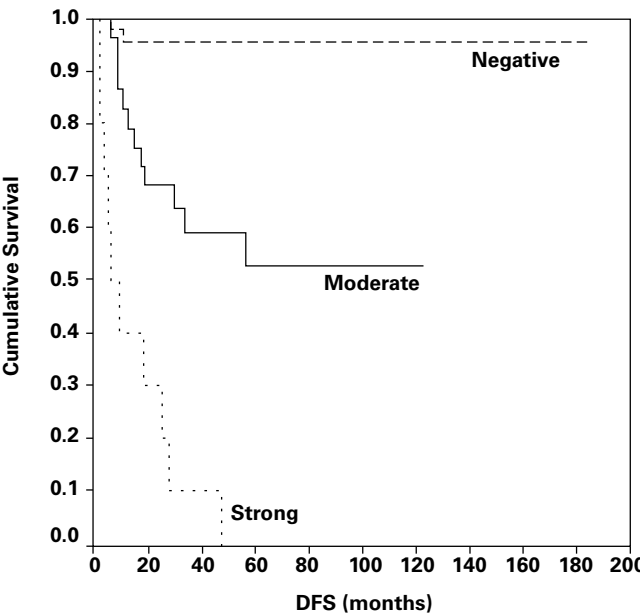


Figure 1 Disease-free survival (DFS) curve stratified by different P-glycoprotein expression levels in intratumoral capillary endothelial cells. Dotted line, strong expression; solid line, moderate expression; dashed line, negative results.

of chemotherapy on MDR1 messenger RNA synthesis, as described in some pretreated neuroblastomas.^{23,24} On the other hand, since chemotherapy usually suppresses the blastematos component, increased P-gp positivity could simply reflect the relative prominence of residual, more immunoreactive areas, such as epithelial and rhabdomyoblastic elements.

We observed the presence of P-gp in endothelial cells of newly formed capillaries in 43 (46%) of 93 tumors examined.

This feature, described in tumors of the central nervous system²⁵⁻²⁹ and in some pretreated bladder carcinomas,³⁰ was a novel finding in nephroblastoma.

P-gp is physiologically present in endothelium of brain, testis, and placental vasculature,⁸ likely functioning as protection from circulating toxic agents. In newly formed intratumoral capillaries, it might contribute to chemoresistance by creating a blood-tumor barrier and interfering with the correct interaction of chemotherapeutic agents with neoplastic cells.²⁵⁻³⁰

In our study, treatment failure and worse outcome paralleled increased endothelial P-gp expression. Tumor angiogenesis results from expansion of existing vessels by sprouting and penetration into a developing neoplasm. How P-gp expression had been induced in the endothelium of capillaries originating from renal vasculature (a district that normally does not express it) was unclear. Previous chemotherapy, as observed for tumor cells, might have promoted it. We noticed an increase in the proportion of vessels expressing P-gp in pretreated tumors, although the correlation was not statistically significant. As endothelial cells of extratumoral vessels also were negative for P-gp in pretreated samples, some intratumoral stimuli might have induced intratumoral capillary endothelium P-gp expression. In fact, it is known that, far from being a simple lining tissue, microvascular endothelium manifests extensive heterogeneity and exhibits diverse functional and biochemical properties that vary among tissues.³¹ Differentiation of endothelial cells is strongly influenced by their microenvironment and may vary in response to growth factors, production of cytokines, secretion of matrix products, production of cell adhesion molecules, and affinity for specific tumor cells.^{32,33} In nephroblastoma, tumoral microenvironment might have an important role in P-gp activation on newly formed capillaries, by means of soluble or paracrine factors. Of course, further structural and functional studies on P-gp expression in endothelial cells are needed, with particular emphasis on interactions with the tumoral microenvironment.

While a direct relationship between the presence of P-gp and the multidrug-resistance phenotype could not be demonstrated clearly in nephroblastoma cells, the causal role of P-gp expressed by the intratumoral vascular component in treatment failure was evident. The activation of this glycoprotein in endothelial cells of tumor vessels should be considered more consistently as one of the factors responsible for the multidrug-resistance phenotype in WT. The proportion of vessels involved may be important in determining the degree of resistance and in designing strategies for overcoming it and increasing therapeutic efficacy.

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References

1. Ueda K, Yoshida A, Amachi T. Recent progress in P-glycoprotein research. *Anticancer Drug Des.* 1999;14:115-121.
2. Thiebaut F, Tsuruo T, Hamada H, et al. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal tissues. *Proc Natl Acad Sci U S A.* 1987;84:7735-7738.
3. Roninson IB. The role of the MDR1 (P-glycoprotein) gene in multidrug resistance in vitro and in vivo. *Biochem Pharmacol.* 1992;43:95-102.
4. Roepe PD. What is the precise role of human MDR 1 protein in chemotherapeutic drug resistance? *Curr Pharm Des.* 2000;6:241-260.
5. Murphy WM, Beckwith JB, Farrow GM. Tumors in infancy and childhood. In: *Tumors of the Kidney, Bladder and Related Urinary Structures.* Washington, DC: Armed Forces Institute of Pathology; 1994:12-92. *Atlas of Tumor Pathology*, Third series, Fascicle 11.
6. Suryanarayan K, Marina N. Wilms' tumour: optimal treatment strategies. *Drugs.* 1998;56:597-605.
7. Ernest S, Rajaraman S, Megyesi J, et al. Expression of MDR1 gene and its protein in normal human kidney. *Nephron.* 1997;77:284-289.
8. Van Kalken CK, Giaccone G, van der Valk P, et al. Multidrug resistance gene (P-glycoprotein) expression in the human fetus. *Am J Pathol.* 1992;141:1063-1072.
9. Beck WT, Grogan TM, Willman CL, et al. Methods to detect P-glycoprotein-associated multidrug resistance in patients' tumors: consensus recommendations. *Cancer Res.* 1996;56:3010-3020.
10. Société Internationale d'Oncologie Pédiatrique. Nephroblastoma Clinical Trial No. 93-01, Protocol June 1993 (revised December 1995). Amsterdam, the Netherlands: SIOP; 1995.
11. Leonardo E, Valente G, Cappia S, et al. Immunohistochemical evaluation of P-glycoprotein in human malignancies by monoclonal antibody MC57. *Int J Cancer.* 1994;57:841-846.
12. Romagnoli G, Poloni F, Flego M, et al. Epitope mapping of the monoclonal antibody MM12.10 to external MDR1 P-glycoprotein domain by synthetic peptide scanning and phage display technologies. *Biol Chem.* 1999;380:553-559.
13. Cianfriglia M, Willingham MC, Tombesi M, et al. P-glycoprotein epitope mapping. I: identification of a linear human-specific epitope in the fourth loop of the P-glycoprotein extracellular domain by MM4.17 murine monoclonal antibody to human multidrug-resistant cells. *Int J Cancer.* 1994;56:153-160.

14. Volm M, Mattern J, Stammers G, et al. Expression of resistance-related proteins in nephroblastoma after chemotherapy. *Int J Cancer*. 1995;63:193-197.
15. O'Meara A, Imamura A, Johnson P, et al. Reactivity of P-glycoprotein monoclonal antibodies in childhood cancers. *Oncology*. 1992;49:203-208.
16. Van Kalken CK, van der Valk P, Hadisaputro MMN, et al. Differentiation-dependent expression of P-glycoprotein in the normal and neoplastic human kidney. *Ann Oncol*. 1991;2:55-62.
17. Oda Y, Rose I, Radig K, et al. Expression of MDR1/p-glycoprotein and multidrug resistance-associated protein in childhood solid tumors. *Virchows Arch*. 1997;430:99-105.
18. Re GG, Willingham MC, el Bahtimi R, et al. Anaplasia and drug selection-independent overexpression of the multidrug resistant gene, *MDR1*, in Wilms' tumor. *Mod Pathol*. 1997;10:129-136.
19. Sola JE, Wojno KJ, Dooley W, et al. P-glycoprotein status of favorable-histology Wilms' tumor predicts treatment outcome. *J Pediatr Surg*. 1994;29:1080-1083.
20. Lizard G, Roignot P, Dusserre-Guion L, et al. Characterization of seven kidney tumors by flow cytometry: analysis of cell cycle, DNA content and P-glycoprotein expression. *Eur Urol*. 1992;21(suppl 1):39-42.
21. Mechetner EB, Schott B, Morse BS, et al. P-glycoprotein function involves conformational transitions detectable by differential immunoreactivity. *Proc Natl Acad Sci U S A*. 1997;94:12908-12913.
22. Sukhai M, Piquette-Miller M. Regulation of the multidrug resistance genes by stress signals. *J Pharm Sci*. 2000;3:268-280.
23. Bourhis J, Bernard J, Hartmann O, et al. Correlation of *MDR1* gene expression with chemotherapy in neuroblastoma. *J Natl Cancer Inst*. 1989;81:1401-1405.
24. Goldstein LJ, Fojo AT, Ueda K, et al. Expression of the multidrug resistance *MDR1* gene in neuroblastoma. *J Clin Oncol*. 1990;8:128-136.
25. Tóth K, Vaughan MM, Peress NS, et al. MDR1 P-glycoprotein is expressed by endothelial cells of newly formed capillaries in human gliomas but not expressed in the neovasculature of other primary tumors. *Am J Pathol*. 1996;149:853-858.
26. Tanaka Y, Abe Y, Tsugu A, et al. Ultrastructural localization of P-gp on capillary endothelial cells in human gliomas. *Virchows Arch*. 1994;425:133-138.
27. Sawada T, Kato Y, Sakayori N, et al. Expression of the multidrug-resistance P-glycoprotein (Pgp, MDR1) by endothelial cells of the neovasculature in central nervous system tumors. *Brain Tumor Pathol*. 1999;16:23-27.
28. Billson AL, Palmer JB, Walker DA, et al. Multidrug resistance gene (*MDR1*) expression in neuro-axial tumours of children and young adults. *Br J Neurosurg*. 1994;8:585-591.
29. Iwahana M, Utoguchi N, Mayumi T, et al. Drug resistance and P-glycoprotein expression in endothelial cells of newly formed capillaries induced by tumors. *Anticancer Res*. 1998;18:2977-2980.
30. Petrylak DP, Scher HI, Reuter V, et al. P-glycoprotein expression in primary and metastatic transitional cell carcinoma of the bladder. *Ann Oncol*. 1994;5:835-840.
31. Yu D, Auerbach R. Brain-specific differentiation of mouse yolk sac endothelial cells. *Brain Res Dev Brain Res*. 1999;117:159-169.
32. Thorin E, Sheeve SM. Heterogeneity of vascular endothelial cells in normal and disease state. *Pharmacol Ther*. 1998;78:155-166.
33. Zetter BR. On target with tumor blood vessels. *Nat Biotechnol*. 1997;15:1243-1247.