Acta Histochemica 115 (2013) 273–278

Contents lists available at SciVerse ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Relation between expression pattern of wild-type p53 and multidrug resistance proteins in human nephroblastomas

Ingrid Hodorová[a], Silvia Rybárová[a], Janka Vecanová[a], Peter Solár[b], Lukáš Plank[c], Jozef Mihalik[a,*]

[a] Department of Anatomy, P.J. Šafárik University, Faculty of Medicine, Šrobárova 2, SK-04180 Košice, Slovak Republic
[b] Department of Cell Biology, P.J. Šafárik University, Faculty of Science, Šrobárova 2, SK-04180 Košice, Slovak Republic
[c] Department of Pathological Anatomy, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Kollárova 2, SK-03659 Martin, Slovak Republic

ARTICLE INFO

Article history:
Received 13 June 2012
Received in revised form 30 July 2012
Accepted 31 July 2012

Keywords:
Pgp/MDR1
MRP1
Wild-type p53
Nephroblastoma
Immunohistochemistry

ABSTRACT

One of the best characterized resistance mechanisms of human cancer is multidrug resistance (MDR) mediated by P-glycoprotein (Pgp/MDR1) and multidrug-resistant related protein (MRP1). In addition to Pgp/MDR1 and MRP1, p53 inactivation or mutation might play a relevant role in therapeutic failure. This study involved 25 children (17 girls and 8 boys) aged 7 months to 10 years treated for unilateral Wilms' tumor. 25 tissue samples of Wilms' tumor and 5 samples of normal human kidneys were obtained from the Department of Pathological Anatomy, Jessenius Faculty of Medicine in Martin, Slovak Republic. We used an indirect immunohistochemical method to determine expression of Pgp/MDR1, MRP1 and wild-type p53 in 25 tissue samples of nephroblastoma. The minority of nephroblastoma specimens showed positivity for both MDR proteins, as well as for wild-type p53. 24% of tissue samples revealed positive results for Pgp/MDR1, 48% for MRP1 and 8% for wild-type p53. Furthermore, our study showed a statistically significant difference between p53 and MRP1 protein expression ($p < 0.01$), but not between p53 and Pgp/MDR1 ($p > 0.05$). No correlation was found between the expression of both multidrug resistance proteins (Pgp/MDR1 and MRP1) and the expression of wild-type p53. Immunohistochemical detection of the expression of MDR proteins and wild-type p53 at the time of diagnosis might assist in choosing specific chemotherapeutics to improve prognosis and therapy.

Introduction

Nephroblastoma is the most common pediatric tumor of the kidney (Breslow et al., 1993). It arises from metanephric blastemal cells and recapitulates renal embryogenesis. In Europe, patients are treated according to the International Society of Pediatric Oncology Protocol (SIOP), which consists of preoperative and surgical resection followed by postoperative treatment (Wittmann et al., 2008). Chemotherapy with vincristine, actinomycin D, and doxorubicin improves the prognosis for the majority of nephroblastomas (Green et al., 1996). A subset of tumors, however, fails to respond to chemotherapy with fatal consequences for patients. A key factor in such chemotherapeutic failures is generally considered to be intrinsic or acquired drug resistance. To date, a large number of mechanisms for acquired multidrug resistance involving proteins responsible for protection of cells and tissues against a number of anticancer agents have been described. Considerable attention has been directed to Pgp/MDR1 overexpression, which is a widely studied phenomenon in many blood and solid tumors and which is responsible for the resistance to chemotherapy. This cell-surface energy-dependent pump reduces the intracellular accumulation of hydrophobic cationic or neutral natural compounds such as anthracyclines, vinca alkaloids, epipodophyllotoxins and paclitaxels (Sola et al., 1994). MRP1 is another transporter protein and belongs to the ABC superfamily as Pgp/MDR1. MRP1 protein transports lipophylic glutathione, glucuronate and sulfate anion conjugates (Efferth et al., 2001). Although the role of Pgp/MDR1 and MRP1 in the pathogenesis of drug resistance is clear, their role as prognostic factors in nephroblastoma is still doubtful.

Mutations or deletion of suppressor gene p53 are the most common genetic abnormalities occurring during cancerogenesis in the majority of cases of human neoplasm. p53 gene, localized on the short arm of chromosome 17 (17p13), encodes nucleic phosphoprotein, and affects several cell functions (induction of many genes, the regulation of the cellular cycle and apoptosis control) (Carson, 1995). Under the condition of mutation of p53 gene, cancer cells remain intact and survive. The index of p53 expression is not an independent prognostic factor in nephroblastoma in children, but it may be helpful in the identification of high risk and low risk patients (Skotnicka-Klonowicz et al., 2001).

In this study, we analyzed the expression of Pgp/MDR1, MRP1 and wild-type p53 in a series of samples in human nephroblastoma.

* Corresponding author.
E-mail address: jozef.mihalik@upjs.sk (J. Mihalik).

0065-1281/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.acthis.2012.08.001

Table 1
Tumor characteristics of patients.

Characteristics		
Sex	Female	18
	Male	7
Age	≤3	7
	>3	18
Preoperative	Yes	22
chemotherapy	No	3
Histopathological type	Low	2
(degree of malignancy)	Intermediate	21
	High	2
Stage of advancement	I	21
	II	3
	III	1
	IV	0
Metastases	Yes	3
	No	22

Furthermore, we decided to determine whether expression of wild-type p53 influences the expression of multidrug resistance proteins (Pgp/MDR1, MRP1).

Materials and methods

Patients

The protocols for the research project were approved by the Ethics Committee at the Šafárik University and conform to the provisions of the Declaration of Helsinki. This study involved 25 children (17 girls and 8 boys), aged 7 months to 10 years, treated for unilateral Wilms' tumor. 25 tissue samples of Wilms' tumor and 5 samples of normal human kidneys were obtained from the Department of Pathological Anatomy, Jessenius Faculty of Medicine in Martin. Patients were diagnosed and treated according to the criteria recommended by the SIOP. Tumor characteristics of patients are summarized in Table 1. The tissue samples for examination were taken from kidney tumors removed directly from three children after diagnosis and from twenty-two children after initial chemotherapy. All the tumors had classical triphasic histology, showing epithelial, mesenchymal and blastemal components in varying proportions. Anaplasia was not detected in any nephroblastoma tumor samples.

Antibodies

We used the following primary monoclonal antibodies: mouse anti-MDR1, UIC2 – clone C494 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse anti-MRP1, clone MRPm6 (Chemicon International, Carlsbad, CA, USA) and mouse anti-p53, clone BP53-12 (Alexis Biochemicals, Enzo Biochemicals, Farmingdale, NY, USA).

Immunohistochemical detection of MDR1, MRP1 and p53

After paraffin removal, sections were heated for different periods of time in a water bath in sodium citrate buffer 0.1 M (pH 6.0) for antigen retrieval. The slides stained for MDR1 and p53 were pretreated in a microwave oven 2 × 5 min, MRP1 slides for 20 min. MDR1 and p53 staining procedure continued by blocking non-specific staining with milk buffer (5% dry milk in Tris buffer) for 30 min at room temperature (RT). In the case of MRP1, the blocking serum was omitted. The next step was application of primary antibodies, which were applied overnight in a humidified chamber at 4 °C. After rinsing in PBS-Tw (3 × 5 min) the sections were subsequently incubated with the secondary antibodies: prediluted biotinylated horse antibody for all those proteins (Vector Laboratories, Burlinghame, CA, USA) for 30 min at RT.

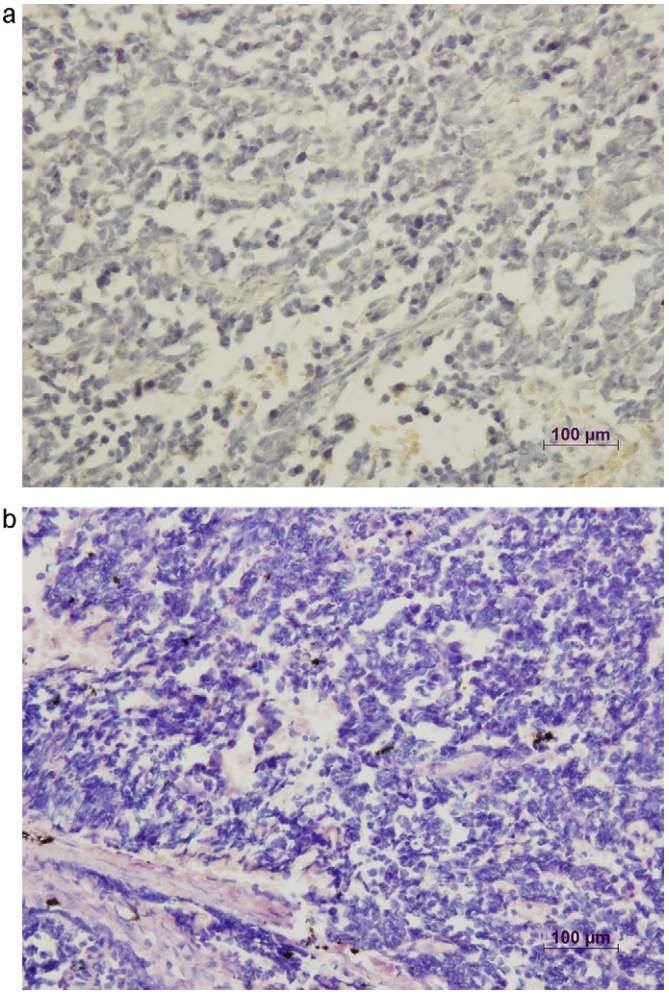

Fig. 1. Nephroblastoma. (A) Negative controls were obtained by omitting the primary antibody. (B) Samples stained by hematoxylin and eosin to provide some general orientation.

The slides were washed with PBS-Tw and submitted to application of peroxidase-conjugated streptavidine: prediluted R.T.U Vectastain for all proteins (Vector Laboratories) for 30 min at RT. The sections were visualized with DAB (3.3′-diaminobenzidine tetrahydrochloride) at a concentration of 0.5 mg/ml in Tris buffer (pH 7.6) and 0.015% H_2O_2. Slides were stream-rinsed with tap water, counterstained with hematoxylin for 2 min, washed in tap water, dried, mounted and coverslipped. Sections processed with omission of primary antibody served as negative control of immunohistochemical procedure (Fig. 1A). The samples stained by hematoxylin and eosin provided some general orientation (Fig. 1B).

Immunohistochemical analysis of MDR1, MRP1 and wild-type p53

Immunostaining was assessed by two independent observers blinded to patient characteristics.

Expression of MDR1, MRP1 and p53 was evaluated separately using the following scale: 3+ = high level (91–100% of positive cells), 2+ = medium level (11–90% of positive cells), 1+ = low level (up to 10% of positive cells), – = negative cells (0% of positive cells). For statistical analysis as positive were considered only samples with high [3+] and medium level [2+] proteins expression. Samples scored as [1+] or [–] were considered negative.