

CLINICAL STUDY

Immunohistochemical detection of MDR proteins in Wilms' tumour

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Abstract: *Objectives:* The aim of our work was to determine the expression of three MDR proteins (MDR1/Pgp, MRP1 and LRP/MVP) in 15 tissue samples of nephroblastoma (Wilms' tumour).

Background: The majority of Wilms' tumours respond well to chemotherapy and are successfully cured, but a small subset displays resistance to therapy. The molecular mechanisms of drug resistance in this tumour type of childhood are still poorly analyzed. In our opinion, the elucidation of reasons for therapy failure in nephroblastomas is urgently needed before cure becomes a reality for children with this cancer.

Methods: To demonstrate these proteins the enzyme indirect immunohistochemical method was used. The brown colour of the diaminobenzidine reaction product allowed us to define the distribution of stain clearly.

Conclusion: Our immunohistochemical analysis did not demonstrate any expression of MDR1 in all cases of nephroblastoma (14 cases were after pre-operative chemotherapy, 1 case wasn't). The analysis of MRP1 and LRP expression in our set revealed 60 % positivity for MRP1 and 26.7 % positivity for LRP. The ability to recognize the multidrug resistance phenotype might assist in choosing specific chemotherapeutic regimens to improve prognosis and therapy (Tab. 2, Fig. 2, Ref. 20). Full Text (Free, PDF) www.bmj.sk.

Key words: immunohistochemistry, nephroblastoma, MDR1/Pgp, MRP1, LRP.

Nephroblastomas (Wilms' tumours) are manageable by surgery and chemotherapy with survival rates above 80 % (Grovas et al, 1997). Although different treatment protocols are used in the United States and in Europe, vincristin, actinomycin D, and doxorubicin have reached wide-spread acceptance as the most active cytostatic drugs for the treatment of nephroblastomas (Green et al, 1996). The majority of Wilms' tumours respond well to chemotherapy and are successfully cured. Even though a small subset displays resistance to therapy. These tumours generally demonstrate the histologic features of anaplasia (nuclear enlargement, hyperchromasia and abnormal mitotic figures). Due to limited patient number, the molecular mechanisms of drug resistance in this tumour type of childhood are still poorly analyzed. In our opinion, the elucidation of reasons for treatment failure in nephroblastomas is urgently needed before cure becomes a reality for children with this cancer.

Concept of the multi-factorial nature of drug resistance have now generally been accepted for many adult cancers (Beck et al, 1998). However, the molecular mechanisms of drug resistance

Tab. 1. Patients and tumour characteristics.

Characteristics	No
All patients	15
Age	≤35 5 ≥4 10
Pre-operative chemotherapy	yes 14 no 1
Pgp (MDR1)	positive 0 negative 15
MRP1	positive 9 negative 6
LRP	positive 4 negative 11

in nephroblastomas are still poorly understood. Over the years, a number of genes were identified to be involved in multidrug resistance (MDR). P-glycoprotein, encoded by the MDR1-gene, the most extensively studied drug transporter, was first described by Ling et al in the 1970s (Gottesmann et al, 2002). Pgp is an ATP-dependent pump that extrudes drugs from cytoplasm and prevents their intracellular accumulation. The multidrug resistance-related protein (MRP1) was first identified in drug resistant lung cancer cells (Cole et al, 1992). Its expression in the outer plasma membrane, in intracellular vesicles, and in the Golgi apparatus speaks for a role as transporter molecule which exports drugs out of the cell and/or sequesters drugs into vesicles.

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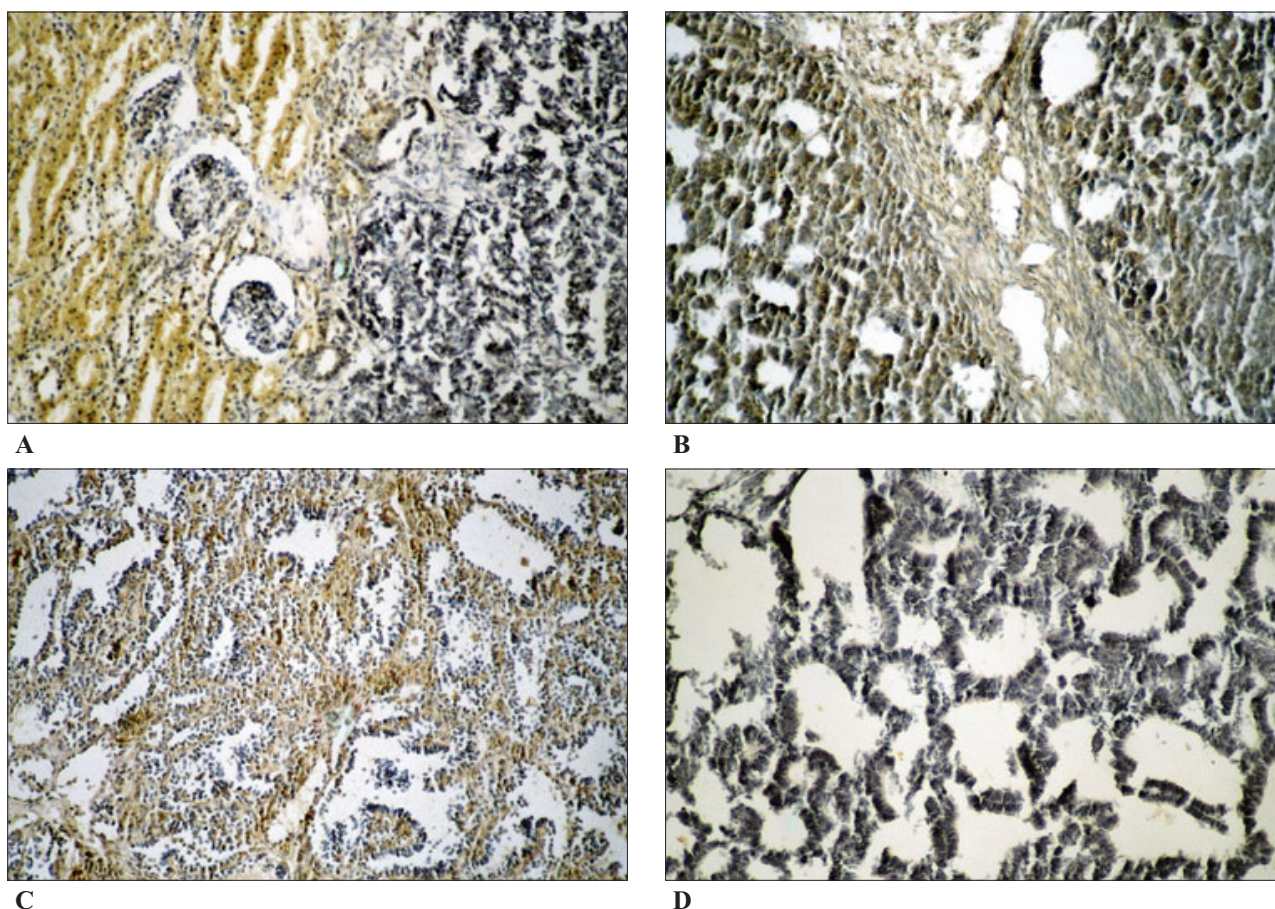


Fig. 1. (A) Boundary line of normal renal tissue with presence of MRP1 and malignant renal tissue with no expression of MRP1. (B) High level of MRP1 expression. (C) Strong expression of LRP. (D) MDR1 protein was not expressed in samples of Wilms' tumour.

Treatment of tumour cells with drugs of the MDR-type induces the expression of MDR1/Pgp and MRP genes (Zhou et al, 1995). Recently, a Mr 110 kDa vesicular protein has been found in multidrug-resistant and Pgp negative lung cancer cells, called lung resistance protein (LRP). LRP has striking homologies to the major vault proteins (Scheffer et al, 1995; Rybárová et al, 2001). Vaults are large ribonucleoprotein complexes consisting of a major vault protein (MVP), three minor proteins and a small RNA molecule. Ultra-structural analyses suggest that vaults may be involved in nucleo-cytoplasmic transport processes (Kedersha et al, 1990). Our previously reported results of MDR1/Pgp, MRP1 and LRP/MVP expression in normal kidney tissue and renal cell carcinomas indicates that these proteins may also be expressed in nephroblastomas.

The aim of the present study was to analyze the expression of MDR1/Pgp, MRP1 and LRP/MVP in formalin-fixed paraffin-embedded sections of 15 Wilms' tumours.

Material and methods

Clinical samples. In the study we have used 15 samples of nephroblastoma. The samples were obtained from the Department of Pathological Anatomy, Jessenius Faculty of Medicine

in Martin. Patients and tumour characteristics are summarized in Table 1.

Immunohistochemical detection of MDR1, MRP1 and LRP by indirect enzymatic immunohistochemical method. Formalin fixed, paraffin embedded tissue blocks were cut (7 μ m) and attached to the slides. The slides were processed for immunohistochemistry. Tissue sections were deparaffinized with xylene and rehydrated in decreasing ethanols to water. The slides were finally washed in phosphate-buffered saline containing 0.05 % Tween-20 (PBS-Tw), pH 7.6. Endogenous peroxidase activity was blocked by 0.3 % H_2O_2 in methanol for 30 minutes at room temperature. According to the analyzed protein, sections were pre-treated in citrate buffer solution in the microwave oven differently. The slides stained for MDR1 and LRP were pre-treated in the microwave 2x5 minutes, MRP1 slides for 20 minutes. MDR1 and LRP staining procedure continued by blocking non-specific staining with milk buffer (5 % dry milk in TRIS) for 30 minutes at room temperature. In the case of MRP1 blocking serum was omitted. The next step was application of primary antibodies. We have used the following antibodies: mouse anti-MDR1, clone C219 (Signet Laboratories, Inc.), mouse anti MRP1, clone MRpm6 (Chemicon International, Inc.) and mouse anti-LRP,

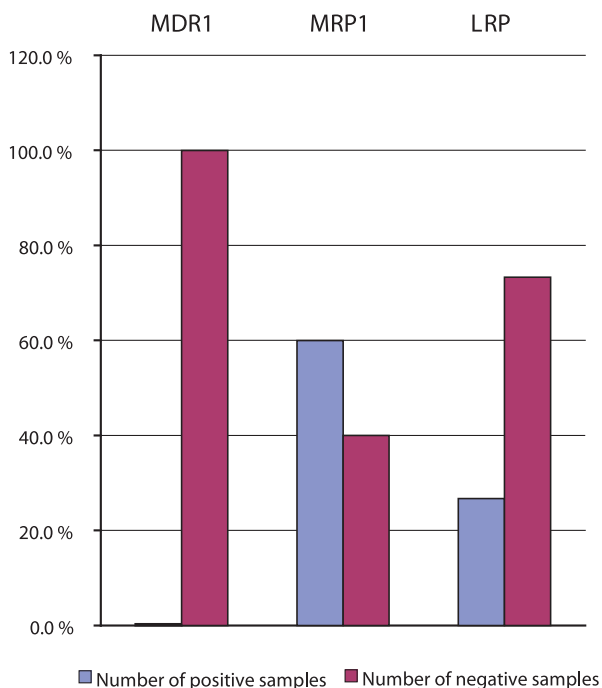


Fig. 2. Comparison of positive and negative tissue specimens of MDR1/Pgp, MRP1 and LRP in nephroblastoma.

LRP56 (BD Transduction Laboratories, USA). Primary antibodies were applied overnight in humidified chamber at 4 °C. After rinsing in PBS-Tw (3x5 minutes) the sections were subsequently incubated with the secondary antibodies: prediluted biotinylated horse antibody for MDR proteins (Vector Laboratories, USA) for 30 minutes at room temperature. The slides were washed with PBS-Tw and submitted to application of peroxidase-conjugated streptavidine: prediluted R.T.U Vectastain for MDR proteins (Vector Laboratories, USA) for 30 minutes at room temperature. The sections stained for MDR proteins were then visualized with DAB (3,3'-diaminobenzidine tetrahydrochloride) at a concentration of 0.5 mg/ml in Tris buffer, pH 7.6 and 0.015 % H₂O₂. Slides were stream-rinsed with tap water, counterstained with hematoxylin for 2 minutes, washed in tap water, dried, mounted and coverslipped. Sections processed with omission of primary antibody served as a negative control of immunohistochemical procedure.

Results

All these samples were immunohistochemically analyzed for MDR1/Pgp, MRP1 and LRP. We have distinguished four categories of quantity of these proteins: 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 level (3+) and medium level (2+) proteins expressions. Samples scored as (1+) or (-) were considered negative.

Tab. 2. Various levels of MDR1/Pgp, MRP1 and LRP/MVP proteins in 15 samples of nephroblastoma.

Quantity of expression	MDR1/Pgp	MRP1	LRP/MVP
3+	0 (0 %)	1 (7 %)	1 (6.7 %)
2+	0 (0 %)	8 (53 %)	3 (20 %)
1+	0 (0 %)	1 (7 %)	3 (20 %)
(-)	15 (100 %)	5 (33 %)	8 (53 %)
Number of positive samples	0 (0 %)	9 (60 %)	4 (26.7 %)
Number of negative samples	15 (100 %)	6 (40 %)	11 (73.3 %)

MDR1/Pgp immunopositivity was observed in none specimen 0 (0 %), all 15 (100 %) tissue samples were negative (Fig. 1D). MRP1 immunoreactivity was found in 9 (60 %) cases of nephroblastoma (Fig. 1C), the rest 6 (40 %) were negative (Fig. 1A). LRP/MVP positive tissue specimens were 4 (26.7 %) (Fig. 1C) and 11 (73.3 %) were negative. Exact results concerning the positivity in all three proteins (in total number of tissue samples and percentage) are shown in Table 2 and Figure 2.

Negative control served as a proof of correct immunostaining methodology, sensitivity of antibodies and other chemicals we have used. In control group no reactivity was observed and no one MDR1/Pgp, MRP1 and LRP positive structure was found.

Discussion

According to our results, the levels of MDR proteins are lower in tumour cells of nephroblastoma than in normal kidney cortex. The overexpression of MDR1 was previously shown to impart broad resistance to a variety of chemotherapeutic drugs (Goldstein et al, 1989). The MDR1 is only expressed in normal human kidney at the apical surface of the proximal tubular epithelial cells and not in other cell types. Our immunohistochemical analysis did not demonstrate any expression of MDR1 in all cases of nephroblastoma (14 cases were after pre-operative chemotherapy, 1 case wasn't). Our findings were supported by O'Meara et al who similarly did not detect MDR1 expression in 12 Wilms' tumours (O'Meara et al, 1992). However, these findings are different from those reported by Sola et al who examined MDR1 in the tissue samples of nephroblastoma by immunogold staining (Sola et al, 1994). The discrepancies in detection of MDR1 in Wilms' tumours may be related to the different time of resection (before or after chemotherapy), various monoclonal antibodies and employed methodology.

MRP1 expression correlated significantly with the survival of patients indicating a prognostic value. These results are in accordance with investigations in other childhood tumours, e.g. neuroblastomas (Norris et al, 1996) as well as adult carcinomas, e.g. of the breast cancer, the lung and the endometrial cancer (Nooter et al, 1997; Oshika et al, 1998; Koshiyama et al, 1999). The drug resistance marker LRP/MVP is also prognostic factor for tumours like ovarian carcinoma, acute myeloid leukemia, multiple myeloma and osteosarcoma (Izquierdo et al, 1996). The analysis of MRP1 and LRP expression in our set revealed 60 %

positivity for MRP1 and 26.7 % positivity for LRP. Generally, these two proteins were more expressed in blastemal and epithelial compartments than in the stromal one (Effert et al, 2001). These results add to the discussion if the different histological components of Wilms' tumour arise from a common clone or not (Pitchard-Jones, 1997). The question is, whether the compartments of nephroblastoma all arise from multipotent renal stem cells or the benign stromal elements are proliferating aberrantly in a malign environment of blastemal and epithelial tumour cells.

Our results indicate that chemotherapeutic pre-treatment induces the expression of MRP1 and LRP/MVP but not MDR1. The ability to recognize the multidrug resistance phenotype and to assess its mechanism at the time of diagnosis might assist in choosing specific chemotherapeutic regimens to improve prognosis and therapy.

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