Virchows Arch (1997) 430:99–105

ORIGINAL ARTICLE

Y. Oda · I. Röse · K. Radig · W. Wagemann
U. Mittler · A. Roessner

Expression of MDR1/p-glycoprotein and multidrug resistance-associated protein in childhood solid tumours

Received: 6 May 1996 / Accepted: 3 July 1996

Abstract We evaluated the expression of MDR1/p-glycoprotein in paediatric tumours using reverse transcriptase polymerase chain reaction (RT-PCR), RNA dot blot analysis, and immunohistochemistry on formalin fixed paraffin-embedded material with JSB-1 and C-219 monoclonal antibodies, and compared these three techniques. The expression of multidrug resistance-associated protein (MRP) gene was examined by RT-PCR assay. We studied MDR1/p-glycoprotein and MRP expression in 13 samples from 10 neuroblastoma patients, 11 samples from 10 nephroblastoma patients, 2 rhabdomyosarcomas, 1 adrenocortical carcinoma and 10 benign tumours or tumour-like lesions. Eleven of 13 neuroblastomas, 7 of 11 nephroblastomas, 2 rhabdomyosarcomas, 1 adrenocortical carcinoma, and 7 of 10 benign tumours or tumour-like lesions showed MDR1 PCR products. By RNA dot blot analysis, MDR1 transcripts were detectable in 11 of 34 specimens. Immunohistochemically, we detected positive reaction products for JSB-1 in 26 of 36 samples. There was a significant correlation between the immunoreactivity for JSB-1 and the expression of MDR1 mRNA expression by RT-PCR (P=0.0001). However, the presence of p-glycoprotein immunostaining does not correlate with the MDR1 expression shown by RT-PCR in every case. As for MRP mRNA expression, 9 of 13 neuroblastomas and 10 of 11 nephroblastomas revealed PCR products.

Y. Oda · I. Röse · K. Radig · A. Roessner (✉)
Department of Pathology,
Otto-von-Guericke University Magdeburg,
Leipziger Strasse 44, D-39120 Magdeburg, Germany
Tel.: (+49) 391-67-15817, Fax: (+49) 391-67-15818

W. Wagemann
Department of Pediatric Surgery,
Otto-von-Guericke University Magdeburg, Germany

U. Mittler
Department of Pediatric Oncology,
Otto-von-Guericke University Magdeburg, Germany

Key words Multidrug resistance · P-glycoprotein · Neuroblastoma · Nephroblastoma · Reverse transcriptase polymerase chain reaction

Introduction

The human MDR1 gene, which encodes the drug efflux pump called p-glycoprotein [26, 36], has been shown to play a major role in the acquisition and maintenance of the multidrug resistance (MDR) phenotype in vitro [16, 31]. Sensitive molecular biological and immunohistochemical techniques have been developed to detect MDR1 mRNA/p-glycoprotein expression in clinical samples of paediatric tumours [3, 9, 22, 30, 32]. An increase in MDR1 transcript levels has already been reported for neuroblastomas by many authors using northern blotting or RNA dot blot analysis [4, 5, 7, 14, 23, 28]. To investigate further association of the expression of the MDR1 gene in childhood cancers with drug resistance, we studied the expression of MDR1/p-glycoprotein in paediatric solid tumours using reverse transcriptase polymerase chain reaction (RT-PCR), RNA dot blot analysis and immunohistochemistry. To find the most sensitive and reliable tools for the assessment of MDR1/p-glycoprotein expression, these three methods were compared. The recently described multidrug resistance-associated protein (MRP) gene, identified by Cole et al. [12], encodes a novel membrane transport protein, the overexpression of which has been associated with a number of non-P-glycoprotein-mediated MDR phenotypes in vitro. Using an RT-PCR assay, the expression of this gene was also examined in this study.

Materials and methods

KB-C1, a drug resistant cell line, and the KB-3-1 parental epidermoid carcinoma cell line (kindly provided by Prof. M. Kuwano, First Department of Biochemistry, Kyushu University, Fukuoka, Japan) were used as standard material for quantification of MDR1 transcript level and p-glycoprotein expression by immunohisto-

Table 1 Amplimers and conditions of thermal cycling for polymerase chain reaction (PCR) amplification (*MRP* multidrug resistance-associated protein, *B2MG* beta 2 microglobulin)

Gene	Length	Primer sequence	Thermal cycling
MDR1	167 bp	5′-CCC ATC ATT GCA ATA GCA GG-3′ 5′-GTT CAA ACT TCT CGT CCT GA-3′	94° C (30 s) 55° C (1 min) 72° C (2 min) 33 cycles
MRP	614 bp	5′-CTG AGA AGG AGG CGC CCT G-3′ 5′-GTG TCC GGA TGG TGG ACT G-3′	94° C (1 min) 57° C (1 min) 72° C (2 min) 30 cycles
B2MG	120 bp	5′-ACC CCC ACT GAA AAA GAT GA-3′ 5′-ATC TTC AAA CCT CCA TGA TG-3′	94° C (30 s) 55° C (1 min) 72° C (2 min) 35 cycles

chemistry. A multidrug resistant mutant, KB-C1, was selected with increasing concentration of colchicine, and was maintained in the presence of 1 μg/ml colchicine [1]. The KB-C1 cell line expressed 270-fold the MDR1 mRNA compared with KB-8, which expressed a minimal level of MDR1 as seen by slot-blot analysis [34].

We examined 13 samples from 10 neuroblastoma patients, 11 specimens from 10 nephroblastoma patients, 2 rhabdomyosarcomas and 1 adrenocortical carcinoma. Furthermore, 10 benign tumours or tumour-like lesions were also investigated. For neuroblastoma, histological subclassification was made according to Hughes et al. [24]. The clinical stages of the disease were determined according to the system of Evans et al. [18]. For nephroblastomas, histological grade and clinical stage were determined according to "Gesellschaft für Pädiatrische Onkologie und Hämatologie" [33] and "Societe Internationale d'Oncologie Pediatrique" [25], respectively. Tissues with necrosis, haemorrhage, or calcification were excluded. Representative tissue was frozen in liquid nitrogen and stored at −80° C for RNA analysis. The remainder of the tissue was fixed in 4% neutral buffered formalin for pathological analysis and immunostaining.

Total RNA was isolated from the tissue specimens and cell lines by the single extraction method as described by Chomczynski and Sacchi [11]. Total RNA was also obtained from peripheral blood mononuclear cells (PBMC) isolated from freshly drawn heparinized normal human blood by centrifugation through Histopaque 1077 and washed with phosphate-buffered saline before lysis. Cellular RNA from PBMC of a healthy volunteer was used for positive control of MRP mRNA [2, 12]. Purity was confirmed on 1% agarose-formaldehyde gel, and quantitation was performed by spectrophotometry analysis.

For reverse transcriptase-polymerase chain reaction (RT-PCR), 1 μg purified cellular RNA was converted to single strand cDNA using a random primer and the Promega (Madison, Wis.) RT system according to the manufacturer's instructions. PCR was performed in a programmable thermal cycler (PTC-100TM: MJ Research, Watertown, Mass.). The oligonucleotides used as amplimers in this study were synthesized by Pharmacia and conditions of thermal cycling were listed in Table 1. cDNA derived from 50 ng of RNA was incubated with 2.5U of *Taq* DNA polymerase (GIBCO) in 50 μl containing 1.5 mM magnesium chloride (0.5 mM for MRP primers), 0.2 mM of each dNTPs and 1×PCR buffer (GIBCO) and 37.5 pmol (25 pmol for MRP) gene specific amplimers. After amplification, 5 μl of each reaction was analysed by electrophoresis on a 4% agarose gel (1% agarose + 3% NuSieve) stained with ethidium bromide, and quantified by laser densitometry. The intensity of each mRNA was standardized with beta 2-microglobulin (B2MG) expression. The level of MDR1 was determined by comparison of the ratio of the intensities of the MDR1 and B2MG PCR products for each samples with the positive control KB-C1 cells (100 units). MDR1 values were estimated as follows: 0 unit, grade 0; <10 units, grade 1; >10 units and <40 units, grade 2; >40 units, grade 3.

The level of MRP mRNA was classified as follows: grade 0, not observed; grade 1, the intensity of the amplified band was less than that of PBMC; grade 2, the intensity was stronger than PBMC.

In order to allow us to perform RNA dot blotting pHDR5A was a kind gift from Dr. M. Gottesman. It consists of a 1.4 kb insert of MDR1 in transcription vector pGEM4 [37]. The plasmid was digested with *Puv*II which cuts midway in the MDR1 cDNA. Digoxigenin (dig)-labelled antisense RNA probe was transcribed using SP6 RNA polymerase with a dig-RNA-labelling kit (Boehringer-Mannheim, Biochemica, Mannheim, Germany) according to the manufacturer's instructions. Serial dilutions of 10, 5 and 1 μg of each sample of denatured total cellular RNA were spotted on the nylon membrane (Hybond N, Amersham) using a BRL Hybridot Manifold apparatus. Samples from both MDR1 negative KB-3-1 and positive KB-C1 cell lines were included on each blot. After fixation by UV-irradiation (UV Stratalinker 2400, Stratagene), the filters were prehybridized for 4 h at 55° C in 50% formamide, 5× standard saline citrate (SSC), 2% blocking reagent (Boehringer-Mannheim), 0.1% N-lauroylsarcosine and 0.02% sodium dodecyl sulphate (SDS). The hybridization was carried out overnight at 55° C in the same solution containing dig-labelled MDR1 antisense RNA probe. After hybridization, the filters were washed with 2× SSC, 0.1% SDS twice for 10 min each at room temperature and then three times with 0.1× SSC, 0.1% SDS for 15 min each at 68° C. The detection of hybridization signals were carried out with DIG nucleic acid detection kit (Boehringer-Mannheim) and chemiluminescent substrates: 1,2-dioxetane chemiluminescent enzyme subtrate (CSPD) (Serva, Heidelberg, Germany). Finally, the membrane was exposed to X-ray film and the hybridization signals were quantified by densitometer. The specificity of the generated cRNA probe was confirmed by northern blotting. Values for MDR1 expression were determined by substrating

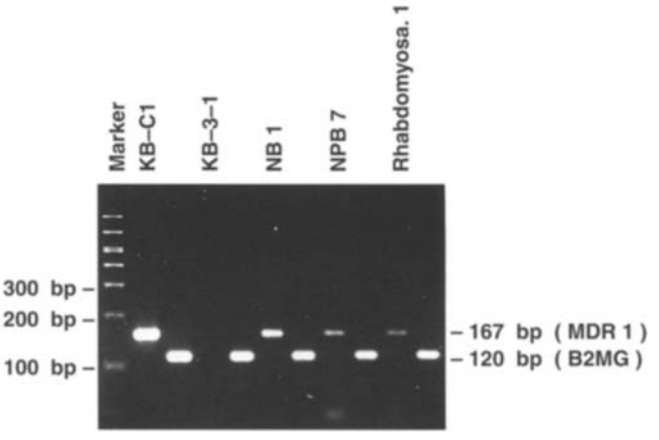

Fig. 1 RT-PCR analysis of MDR1 mRNA expression. The upper band shows the PCR products following amplification of the MDR1 sequence. The lower band shows B2MG-specific PCR products amplified in separate tubes as internal control. Drug resistant cell line, KB-C1 shows strong positive signal. There was no MDR1 PCR product in drug sensitive cell line KB-3-1. (*NB 1* neuroblastoma case 1; *NPB 7* nephroblastoma case 7; Rhabdomyosa. 1: rhabdomyosarcoma case 1. These tumours expressed MDR1 mRNA at high or intermediate levels

Table 2 Patient data and results of MDR1 and MRP expression in paediatric tumours (*CT* chemotherapy, *DB* dot blot, *PGP* p-glycoprotein, *IHC* immunohistochemistry, *RT-PCR* reverse transcriptase-PCR, *M* months, *y* years, *d* days, *M* male, *F* female, *NB* neuroblastoma, *NPB* nephroblastoma, *NE* not examined)

Case number	Age/ Sex	Histological diagnosis	Prior CT	Grade[a]	Stage	MDR1 RT-PCR	DB	PGP JSB-1	IHC C-219	MRP RT-PCR	Prognosis (m)
1	9m/F	NB	No	3	IV-S	3	3	1	0	2	Alive (84)
2	72m/F	NB	No	3	IV	3	2	2	1	0	Dead (12)
2′		Recurrence	Yes			3	1	2	1	0	
3	5m/F	NB	No	3	IV-S	0	0	NE	NE	1	Dead (11)
3′		Recurrence	Yes			2	0	0	0	1	
4	72m/F	NB	Yes	2	IV	3	0	1	0	0	Dead (7)
5	12m/F	NB	Yes	3	III	3	0	2	1	1	Aive (72)
6	36m/M	NB	No	3	III	3	1	1	0	2	Alive (16)
7	10m/F	NB	No	3	II	3	3	0	NE	1	Alive (12)
8	24m/F	NB	Yes	3	IV	0	0	0	0	0	Alive (5)
9	5m/M	NB	No	3	I	1	0	1	0	1	Alive (2)
10	5m/F	NB	Yes	3	IV	1	0	2	1	1	Dead (2)
10′		Liver metastasis	Yes			3	3	2	2	2	
11	96m/M	NPB	No	S	II	2	0	2	1	2	Alive (84)
12	72m/M	NPB	No	S	IV	2	0	1	1	2	Alive (70)
13	48m/M	NPB	No	S	II	0	0	0	0	1	Alive (63)
14	47m/M	NPB	No	S	II	0	0	0	0	1	Dead (6)
15	15m/M	NPB	Yes	H	III	2	0	1	0	1	Dead (18)
15′		Recurrence	Yes			0	0	0	0	1	
16	60m/F	NPB	Yes	S	I	0	0	0	0	1	Alive (54)
17	42m/M	NPB	Yes	H	III	2	NE	1	1	0	Dead (8)
18	48m/M	NPB	Yes	S	IV	3	2	1	0	1	Alive (24)
19	12m/M	NPB	Yes	S	II	3	2	1	1	2	Alive (22)
20	96m/M	NPB	Yes	S	III	3	0	2	1	1	Alive (24)
21	3y/M	Rhabdomyosarcoma				2	NE	2	1	1	Dead (12)
22	17y/F	Rhabdomyosarcoma				1	0	1	0	1	Alive (48)
23	8y/F	Adrenocortical carcinoma				3	1	2	1	2	Dead (6)
24	3y/F	Lipoblastoma				0	0	0	0	1	
25	8m/M	Lipoblastoma				0	0	0	0	2	
26	10d/M	Haemangioendothelioma				1	0	0	0	1	
27	15y/M	Haemangioendothelioma				2	0	1	1	1	
28	14y/M	Paraganglioma				3	3	1	1	2	
29	14y/M	Ganglioneuroma				2	1	2	2	1	
30	7y/F	Neurofibroma				1	NE	2	1	0	
31	3y/F	Plasma cell granuloma				1	0	1	0	2	
32	5y/M	Plasma cell granuloma				1	0	1	0	1	
33	5y/F	Eosinophilic granuloma				0	0	1	0	0	

[a] For neuroblastomas the grade is given according to Hughes [24]. For nephroblastomas the grade in that according to Gesellschaft für Pädiatrische Onkologie und Hämatologie [33] with *S* indicating standard risk and *H* indicating high risk

the value obtained in the negative control line KB-3-1, and then normalizing all samples to a value of 100 units for 10 µg total RNA of the positive control line KB-C1. The negative signals were scored as grade 0. The value less than 3 units was considered to be a low expression, grade 1; 3–10 units intermediate, grade 2; more than 10 units high, grade 3.

For immunohistochemistry formalin-fixed, paraffin-embedded pellets of KB-3-1 and KB-C1 cell lines were used as controls. Sections were cut at 4 µm from paraffin-embedded samples and pellets of cell lines and were dewaxed. After inhibition of endogenous peroxidase, tissue sections were exposed to JSB-1 monoclonal antibody (Crawly, England; dilution 1:20) and C-219 murine monoclonal antibody (Centocor, Malvern, Pa.; dilution 1:5) for 16 h at 4° C. A secondary horse anti-mouse antibody conjugated with biotin was followed by streptavidin. The colour reaction was developed in diaminobenzidine solution for 10 min and sections were counterstained with haematoxylin. A section without the primary antibody served as a negative control. The slides were reviewed by three pathologists independent of other experimental results and scored as negative, grade 0; <50% of cells showing staining, grade 1; >50% of cells showing immunoreactivity, grade 2.

The correlation between RT-PCR and immunohistochemistry in MDR1 expression was evaluated by chi-square test. For testing the effect of MDR1 and MRP expression on the prognosis, Kaplan-Meier plots were constructed and Wilcoxon-test was performed. A P value less than 0.05 was considered significant.

Results

RT-PCR for MDR1

The feasibility of RT-PCR for MDR1 was confirmed with the cell lines. The drug sensitive KB-3-1 showed no MDR1 product, whereas a strong positive signal was obtained with the drug resistant cell line KB-C1 (Fig. 1). Out of the 37 specimens, 28 (76%) had positive PCR products. For neuroblastomas, 11 of 13 specimens showed MDR1 expression (Table 2 and Fig. 2A). Four of five chemotherapy free samples revealed MDR1 expres-

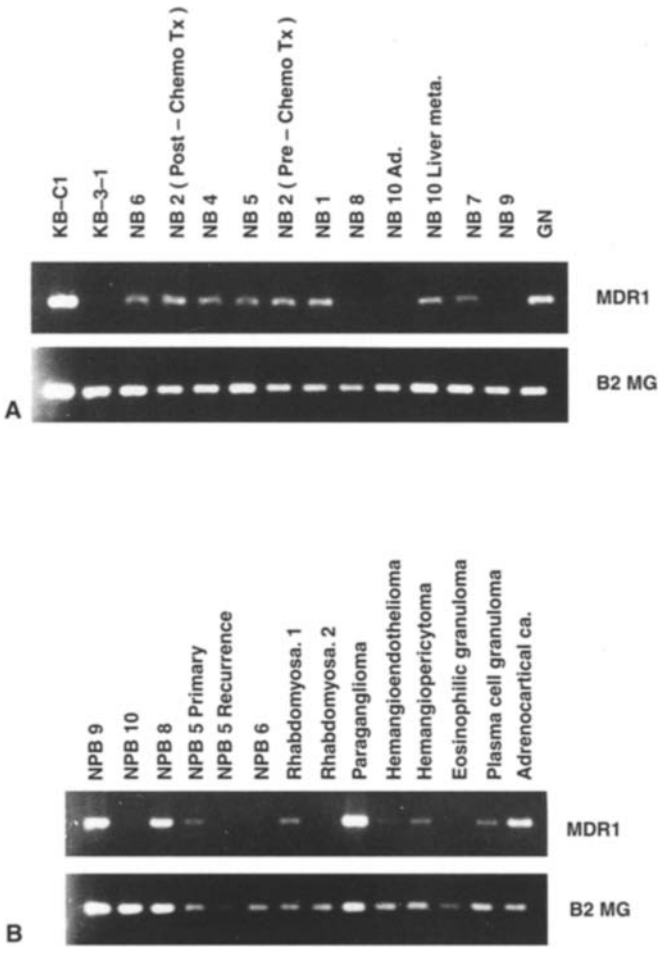

Fig. 2 **A** PCR analysis for MDR1 and B2MG gene expression in NBs. NB2 revealed MDR1 expression at a high level before and after chemotherapy. *Post-Chemo Tx* post chemotherapy, *Ad.* adrenal tumour, *meta.* metastasis, *GN* ganglioneuroma). **B** RT-PCR for MDR1 in NPBs and other tumours or tumour-like lesions. Paraganglioma and adrenocortical carcinoma showed MDR1 expression at a high level

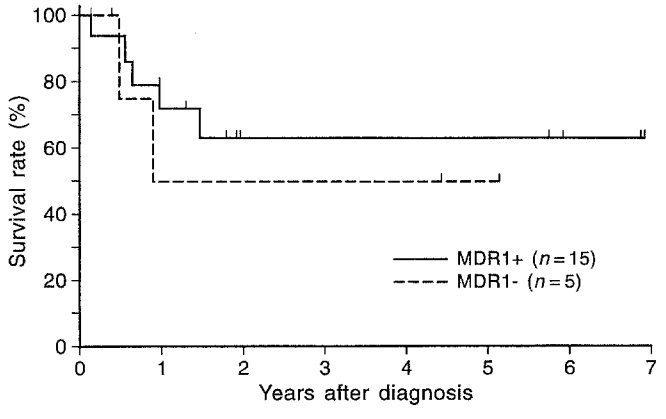

Fig. 3 Correlation between survival according to the MDR1 expression by RT-PCR in ten NBs and ten NPBs

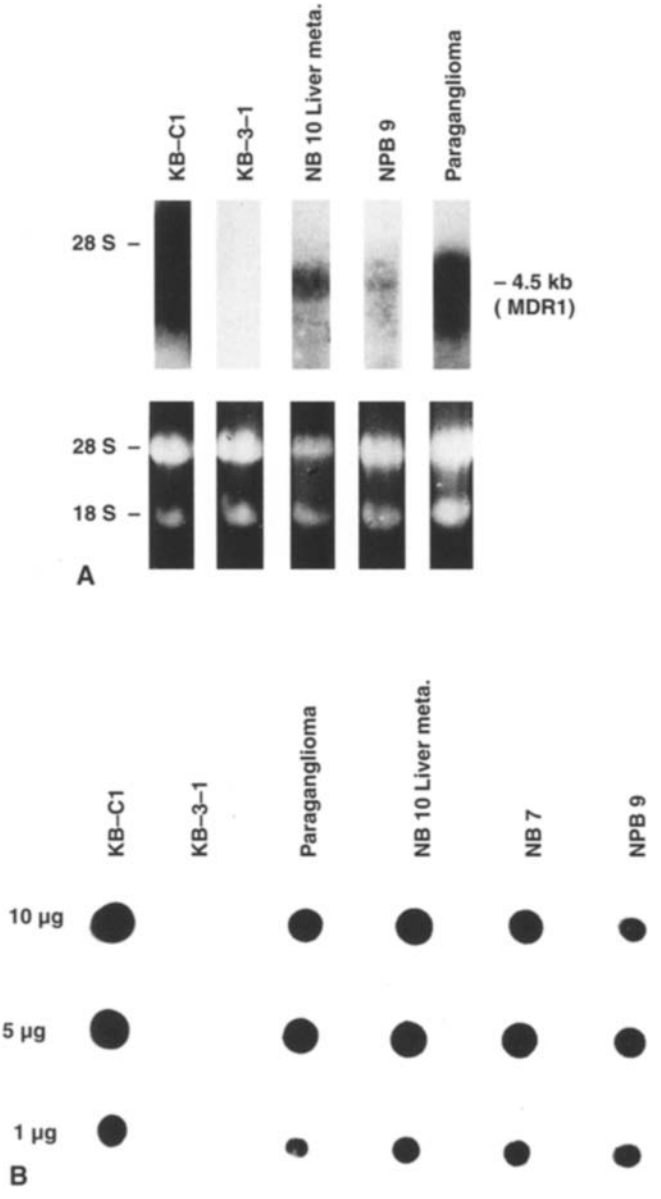

Fig. 4 **A** Specific detection of the 4.5 kb MDR1 mRNA by northern blotting using digoxigenin-labelled cRNA probe. The agarose gel is coloured with ethidium bromide showing the quality of ribosomic RNAs 28S and 18S. **B** DB analysis for the MDR1 transcripts in paediatric tumours. MDR1 mRNA levels of the multidrug-resistant KB-C1 cell, the drug sensitive KB-3-1 cell and clinical specimens were determined by densitometry

sion initially. Two cases had matched tumours before and after chemotherapy. In case 3, MDR1 expression was recognized only after chemotherapy but in case 2, both pre and post chemotherapy specimens expressed MDR1 at high level.

In the nephroblastomas, 7 of 11 samples revealed MDR1 expression at intermediate or high levels (Table 2 and Fig. 2B). Two of four untreated tumours showed MDR1 expression. There was no significant relationship between MDR1 expression. There was no significant relationship between MDR1 expression and prognosis in

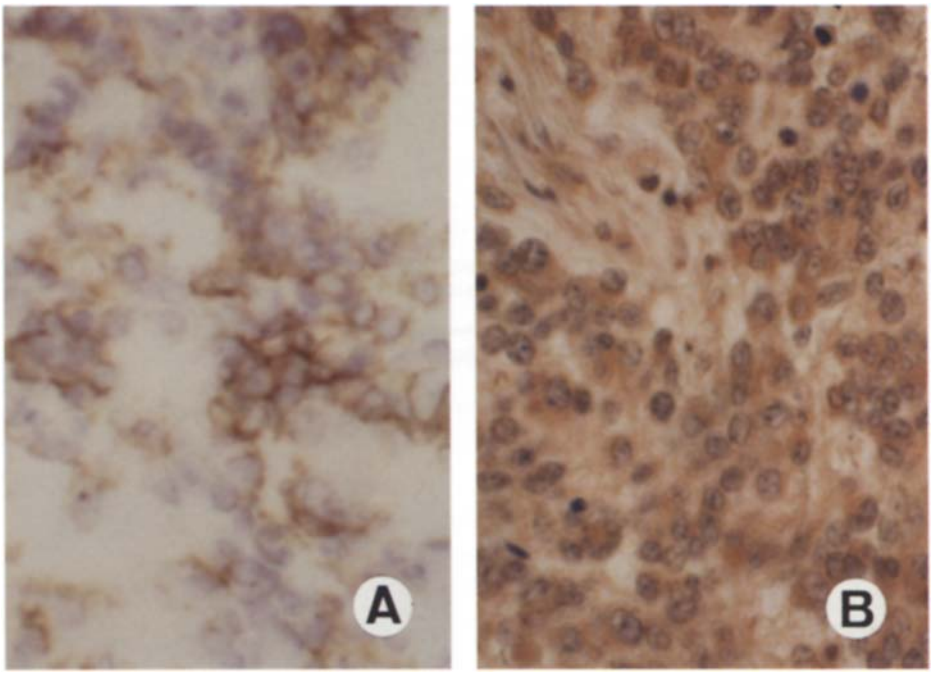

Fig. 5 **A** Drug resistant KB-C1 cells shows positive immunoreaction for JSB-1 monoclonal antibody along the plasma membrane. **B** In NB 10, tumour cells show multifocal positivity of the cytoplasmic membrane and Golgi area with JSB-1 (avidin-biotin peroxidase complex method ×400)

our neuroblastoma and nephroblastoma series (Fig. 3: P=0.131). In other tumours, one adrenocortical carcinoma and one paraganglioma showed high MDR1 expression, compared with other types of tumour or tumour-like lesions (Table 2 and Fig. 2B).

Dot blot analysis of MDR1 expression

The specificity of cRNA probe and dot blot analysis was verified by northern blotting (Fig. 4A). We examined 34 specimens and MDR1 transcripts were detectable in 11 samples (32%). Among 11 positive specimens, 6 were from 13 neuroblastoma specimens, 2 from 10 nephroblastomas and 3 from 11 other tumours or tumour-like lesions (Table 2 and Fig. 4B). Out of these 11 dot blot positive cases, 10 showed a high level of MDR1 expression by RT-PCR and 1 showed an intermediate level. There were no dot blot positive cases in which RT-PCR showed no MDR1 gene product.

Immunohistochemical detection of p-glycoprotein

Paraffin-embedded cell pellets were used as control. Drug resistant KB-C1 shows positive reaction products in the plasma membrane, while parental KB-3-1 revealed no immunoreactivity for JSB-1 or C-219 (Fig. 5A). Of 36 specimens, 26 (72%; 6/13 neuroblastomas, 7/11 nephroblastomas, 2/2 rhabdomyosarcomas, 1/1 adrenocortical carcinoma and 7/10 benign tumours or tumour-like lesions) showed positive reaction for JSB-1 antibody (Table 2 and Fig. 5B). However, 16 of 35 (46%; 5/11 neuroblastomas, 5/11 nephroblastomas, 1/2 rhabdomyosarcomas, 1/1 adrenocortical carcinoma and 4/10 benign tumours or tumour-like lesions) revealed immunoreactivity for C-219 (Table 2). Concordance between RT-PCR, dot blot and immunohistochemistry with JSB-1 was found in 17 samples (47; Table 3). Nevertheless, concordance between the positivity of MDR1 expression by RT-PCR and p-glycoprotein expression by immunohistochemistry with JSB-1 was recognized in 33 (92%) of 36 samples (Table 2). Two neuroblastomas and one haemangioendothelioma showed negative reaction for JSB-1 despite the positivity of RT-PCR. There was a significant correlation between the immunoreactivity for JSB-1 and the expression of MDR1 mRNA by RT-PCR (chi-square test: P=0.0001).

Table 3 Comparison of MDR1/PGP expression using RT-PCR, DB analysis, and IHC (JSB-1) in 33 paediatric tumours or tumour-like lesions

Number of samples	RT-PCR	DB	IHC
10	+	+	+
1	+	+	−
12	+	−	+
2	+	−	−
1	−	−	+
7	−	−	−

RT-PCR for MRP

Of 37 samples, 31 (84%) showed MRP positive products. In the neuroblastomas, 9 of 13 samples revealed MRP expression (Fig. 6). In two matched neuroblastomas, no change was recognized at the levels of MRP before and after chemotherapy. In the nephroblastomas, 10 of 11 specimens had positive products. There was no sig-

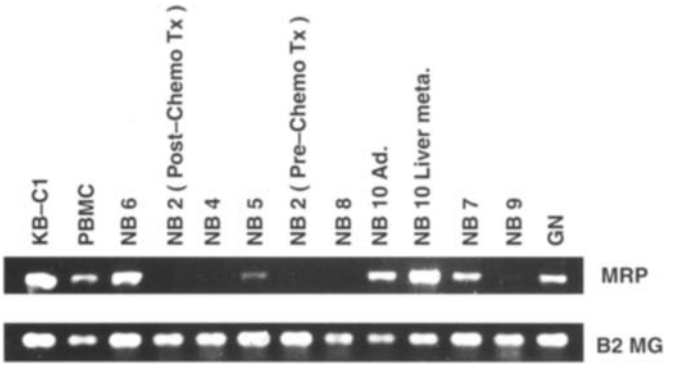

Fig. 6 Analysis of MRP mRNA and B2MG expression in NBs using RT-PCR. Total RNA from PBMC was used as control

nificant relationship between MRP expression and prognosis in neuroblastomas or nephroblastomas (P=0.473).

Discussion

Recent studies have revealed high levels of MDR1/p-glycoprotein in some normal tissue including kidney, colon, adrenal gland and liver [19, 29]. The tumour arising from these tissues also shows high levels of MDR1 [22]. Expression of MDR1/p-glycoprotein has also been documented in neuroblastoma [3, 32]. Two authors [7, 23] using RNA dot blot analysis on neuroblastoma samples showed that a significantly greater number of treated versus untreated cases expressed higher levels of MDR1 mRNA, suggesting that the acquisition of a multidrug resistant phenotype is related to chemotherapy. In our study, there was no significant correlation between MDR1 mRNA expression and therapy status or prognosis, possibly due to the small number of cases.

For the evaluation of MDR1 mRNA expression, Brophy et al. [8] recommended RT-PCR in comparison with RNA dot blot analysis, in situ hybridization and immunohistochemistry, because of its relative simplicity and specificity. Apart from Bordow et al. [6], no investigator has studied the MDR1 expression in paediatric tumours using RT-PCR. In this study, 28 of 37 specimens (76%) showed MDR1 expression by RT-PCR, while 11 of 34 specimens (32%) revealed MDR1 mRNA expression by dot blot analysis even when employing a cRNA probe which is considered to be more sensitive than a cDNA probe. We could not detect the MDR1 transcript by dot blot in 14 samples which showed positive PCR product. Therefore, we consider that RT-PCR is more sensitive than dot blot analysis to detect MDR1 mRNA.

Generally, nephroblastoma is a highly chemoresponsive tumour [15], and infrequent MDR1 expression was previously demonstrated by RNA dot blot analysis [22]. However, 7 of 11 specimens revealed MDR1 expression by RT-PCR in the current study, whereas the MDR1 transcript was demonstrated in only 2 specimens by RNA dot blot analysis. Our results suggest that nephroblastomas of childhood are not necessarily all chemoresponsive tumours and the clinical significance of a low level of MDR1 expression should be investigated within large systematically-treated patient groups by employing sensitive techniques such as RT-PCR.

In this study, formalin-fixed paraffin-embedded clinical materials were examined for the presence of p-glycoprotein by immunohistochemical staining using two monoclonal antibodies, JSB-1 and C-219. We tried to use C-219 for paraffin-embedded sections, but the immunoreactivity was weaker than JSB-1. Kandel et al. [27] described that C-219 antibody from a different lot number decreased the number of cases which showed positive immunostaining. Furthermore, Cordon-Cardo et al. [13] recommended using the C-219 antibody only for frozen sections because the determinants recognized by C-219 are irreversibly masked or denatured through formalin fixation and processing. Several investigators have pointed out that applying immunohistochemistry alone is insufficient and must be supplemented by additional assays such as northern blotting and quantitative RT-PCR [17]. Therefore, careful interpretation of the immunohistochemistry of p-glycoprotein should be done.

MRP, a gene recently isolated from a non-p-glycoprotein-mediated multidrug-resistant small cell lung cancer, is a candidate multidrug-resistance gene [12]. It is considered to encode a 190 kDa transporter membrane protein [2, 12]. It is still unclear whether MRP mRNA expression is associated with drug resistance in vitro. Some authors have found no correlation between MRP expression and multidrug resistance in vitro [10, 20]. In the in vivo study, however, some authors have found a contribution of MRP mRNA expression to the multidrug resistance of haematopoietic neoplasms [21] and thyroid cancer [35]. Recently, Bordow et al. [6] demonstrated the MRP mRNA expression in 5 human neuroblastoma cell lines and in 25 primary neuroblastomas. In our study, 9 of 13 neuroblastomas (69%) and 10 of 11 nephroblastomas (90%) showed MRP expression.

This study confirms the usefulness of RT-PCR to detect the MDR1 mRNA in paediatric tumours. The clinical correlation with MDR1 expression shown by RT-PCR should be demonstrated in a large number of systematically-treated patient groups.

Acknowledgements The authors are grateful to Dr. Michael Gottesman, the Laboratory of Molecular Biology, National Institute of Health, Bethesda, Md., for providing the probe pHDR5A. Also, we thank Prof. Michihiko Kuwano, First Department of Biochemistry, Kyushu University, Fukuoka, Japan for the donation of KB-3-1 and KB-C1 cell lines. We thank Mr. B. Wusthoff for editing the manuscript. Yoshinao Oda is granted an Alexander-von-Humboldt Fellowship. This study was supported by Deutsche Forschungsgemeinschaft.

References

1. Akiyama S, Fojo A, Hanover JA, Pastan I, Gottesman MM (1985) Isolation and genetic characterization of human KB cell lines resistant to multiple drugs. Somatic Cell Genet 11:117–126

2. Barrand MA, Heppel-Parton AC, Wright KA, Rabbitts PH, Twentyman PR (1994) A 190-kilodalton protein overexpression in non-p-glycoprotein-containing multidrug-resistant cells and its relationship to the MRP gene. J Natl Cancer Int 86: 110–117
3. Bates SE, Shieh CY, Tsokos M (1991) Expression of mdr1/P-glycoprotein in human neuroblastoma. Am J Pathol 139:305–315
4. Benard J, Bourhis J, Riou G (1991) Activation of N-myc oncogene and associated genes to chemoresistance, prognostic value for neuroblastoma. Bull Cancer (Paris) 78:91–97
5. Benard J, Bourhis J, de-Vathaire F, Ferrandis E, Terrier-Lacombe MJ, Lemerle J, Riou G, Hartmann O (1994) Prognostic value of MDR1 gene expression in neuroblastoma: results of a multivariante analysis. Prog Clin Biol Res 385:111–16
6. Bordow SB, Haber M, Madafiglio J, Cheung B, Marshall GM, Norris MD (1994) Expression of the multidrug resistance-associated protein (MRP) gene correlates with amplification and overexpression of the N-myc oncogene in childhood neuroblastoma. Cancer Res 54:5036–5040
7. Bourhis J, Benard J, Hartmann O, Boccon-Gibod L, Lemerle J, Riou G (1989) Correlation of MDR1 gene expression with chemotherapy in neuroblastoma. J Natl Cancer Inst 81:1401–1405
8. Brophy NA, Marie JP, Rojas VA, Warnke RA, McFall PJ, Smith SD, Sikic BI (1994) Mdr1 gene expression in childhood acute lymphoblastic leukemias and lymphomas: A critical evaluation by four techniques. Leukemia 8:327–335
9. Chan HSL, Haddad G, Thorner PS, DeBoer G, Lin YP, Ondrusek N, Yeger H, Ling V (1990) P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. N Engl J Med 325:1608–1614
10. Chen G, Jaffrezou J-P, Fleming WH, Duran GE, Sikic BI (1994) Prevalence of multidrug resistance related to activation of the mdr1 gene in human sarcoma mutants derived by single-step doxorubicin selection. Cancer Res 54:4980–4987
11. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159
12. Cole SPC, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AMV, Deeley RG (1992) Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 258:1650–1654
13. Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino Jr, Melamed MR (1990) Expression of the multidrug resistance gene product (p-glycoprotein) in human normal and tumor tissues. J Histochem Cytochem 38:1277–1287
14. Corrias MV, Cornaglia-Ferraris P, Di-Martino D, Stenger AM, Lanino E, Boni L, Tonini GP (1990) Expression of multiple drug resistance gene, MDR1, and N-myc oncogene in Italian population of human neuroblastoma patients. Anticancer Res 10:897–902
15. D'Angio GJ, Breslow N, Beckwith B, Evans A, Baum E, DeLimier A, Fernbach D, Hrabovsky E, Jones B, Kelalis P, Othersen B, Tefft M, Thomas PRM (1989) Treatment of Wilms' tumor: Results of the third national Wilms' tumor study. Cancer 64:349–360
16. Dietel M (1991) What's new in cytostatic drug resistance and pathology. Pathol Res Pract 187:892–905
17. Dietel M (1993) Meeting report. Second international symposium on cytostatic drug resistance. Cancer Res 53:2683–2688
18. Evans AE, D'Andio GJ, Randolph JA (1971) A proposed staging for children with neuroblastoma. Cancer 27:374–378
19. Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, Pastan I (1987) Expression of a multidrug-resistance gene in human tumors and tissues. Proc Natl Acad Sci USA 84:265–269
20. Futscher BW, Abbaszadegan Mr, Domann F, Dalton WS (1994) Analysis of MRP mRNA in mitoxantrone-selected, multidrug-resistant human tumor cells. Biochem Pharmacol 47:1601–1606
21. Gekeler V, Beck J, Noller A, Wilisch A, Frese G, Neumann M, Handgretinger R, Ehninger G, Probst H, Niethammer D (1994) Drug-induced changes in the expression of MDR-associated genes: investigations on cultured cell lines and chemotherapeutically treated leukemias. Ann Hematol [Suppl] 69: 519–524
22. Goldstein LJ, Galski H, Fojo A, Willingham M, Lai S-L, Gazdar A, Priker R, Green A, Crist W, Brodeur GM, Lieber M, Cossman J, Gottesman MM, Pastan I (1989) Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst 81:116–124
23. Goldstein LJ, Fojo AT, Ueda K, Crist W, Green A, Brodeur G, Pastan I, Gottesman MM (1990) Expression of the multidrug resistance, MDR1, gene in neuroblastoma. J Clin Oncol 8: 128–136
24. Hughes M, Marsden HB, Palmer MK (1974) Histologic patterns of neuroblastoma related to prognosis and clinical staging. Cancer 34:1706–1711
25. Jereb B, Tournade MF, Jemerle J, Voute PA, Delemarre JF, Ahstrom L, Flamant R, Gerard-Marchant R, Sandstedt B (1980) Lymph node invasion and prognosis in nephroblastoma. Cancer 45:1632–1636
26. Juliano R, Ling A (1976) A surface glycoprotein modulation drug permeability in Chinese hamster ovarian cell mutants. Biochim Biophys Acta 455:152–162
27. Kandel RA, Campbell S, Noble-Topham S, Bell R, Andrulis IL (1995) Correlation of p-glycoprotein by immunohistochemistry with mdr-1 mRNA levels in osteosarcomas. Pilot study. Diagn Mol Pathol 4:59–65
28. Nakagawara A, Kadomatsu K, Sato S, Kohno K, Takano H, Akazawa K, Nose Y, Kuwano M (1990) Inverse correlation between expression of multidrug resistance gene and N-myc oncogene in human neuroblastoma. Cancer Res 50:3043–3047
29. Noonan K, Beck C, Holtzmayer T, Chin JE, Wunder JS, Andrulis IL, Gazdar AF, Willman CL, Griffith B, Von Hoff DD, Roninson IB (1990) Quantitative analysis of MDR1 (multidrug resistance) gene expression in human tumors by polymerase chain reaction. Proc Natl Acad Sci USA 87:7160–7164
30. McDowell H, Peuchmaur M, Dominici C, Flamant F (1993) Multidrug resistance gene transcript level, and p-glycoprotein expression in paediatric malignant mesenchymal tumours. Anticancer Res 13:1863–1866
31. Pastan I, Gottesman M (1987) Multidrug resistance in human cancer. N Engl J Med 316:1388–1393
32. Ramani P, Dewchand H (1995) Expression of mdr1/P-glycoprotein and p110 in neuroblastoma. J Pathol 175:13–22
33. Schmidt D, Harms D (1983) Histology and prognosis of nephroblastoma – with particular reference to special variants. Klin Padiatr 195:214–221
34. Shen DW, Fojo A, Chin JE, Roninson IB, Richert N, Pastan I, Gottesman MM (1986) Human multidrug-resistant cell lines: Increased mdr1 expression can precede gene amplification. Science 232:643–645
35. Sugawara I, Arai T, Yamashita T, Yoshida A, Masunaga A, Itoyama S (1994) Expression of multidrug resistance-associated protein (MRP) in anaplastic carcinoma of the thyroid. Cancer Lett 82:185–188
36. Ueda K, Cornwell MM, Gottesman MM, Pastan I, Roninson IB, Ling V, Riordan JR (1986) The mdr1 gene, responsible for multidrug-resistance, codes for P-glycoprotein. Biochem Biophys Res Commun 141:956–962
37. Ueda K, Clark DP, Chen C, Roninson IB, Gottesman MM, Pastan I (1987) The human multidrug resistance (mdr1) gene: cDNA cloning and tanscription initiation. J Biol Chem 262:505–508