Different expression profiles of Y-box-binding protein-1 and multidrug resistance-associated proteins between alveolar and embryonal rhabdomyosarcoma

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Nuclear expression of the Y-box-binding protein-1 (YB-1) has been reported to regulate the expression of both P-glycoprotein (P-gp) and major vault protein (MVP), and to regulate proliferative activities in human malignancies. Based on morphology and molecular biology, rhabdomyosarcoma (RMS) can be divided into two major types: embryonal type and the more aggressive alveolar type. Thirty-five cases of embryonal RMS (ERMS) and 28 cases of alveolar RMS (ARMS) were examined immunohistochemically for the nuclear expression of YB-1 and the intrinsic expression of P-gp, multidrug resistance (MDR)-associated protein (MRP) 1, 2, and 3, breast-cancer resistant protein (BCRP) and MVP, and the findings were compared with proliferative activities as evaluated by the MIB-1-labeling index (LI). Moreover, mRNA levels of these MDR-related molecules were assessed using a quantitative reverse transcriptase-PCR method in 18 concordant frozen materials. P-gp expression was more frequently observed ARMS, compared with ERMS ($P = 0.0332$), whereas immunoreactivity for BCRP was more frequently recognized in ERMS ($P = 0.0184$). Nuclear expression of YB-1 protein was correlated with P-gp ($P = 0.0359$) and MVP ($P = 0.0044$) expression, and a higher MIB-1-labeling index ($P = 0.0244$) in ERMS, however, in ARMS no such relationships were observed. These immunohistochemical results indicate that different expression profiles of MDR-related molecules and their correlation with YB-1 nuclear expression support the concept that ERMS and ARMS are molecular biologically distinct neoplasms. Apart from ERMS, frequent P-gp expression in ARMS may be independent from YB-1 regulation. However, YB-1 may be a candidate for a molecular target in rhabdomyosarcoma therapy, especially in ERMS. (*Cancer Sci* 2008; 99; 726–732)

Y-box-binding protein-1 (YB-1) has been reported to be as a transcription factor which interacts with the inverted CCAAT-box (Y-box) in promoters and enhancers of multiple genes. YB-1 has been reported to play a critical role in cell proliferation, DNA replication and drug resistance.[1,2] In particular, previous studies have implicated YB-1 as a regulatory factor for the multidrug resistance (MDR)1 gene in human malignancy.[1,2] MDR is a frequent cause of treatment failure in cancer patients. One mechanism of MDR is overexpression of ATP-binding cassette (ABC) transporter proteins that function as a drug efflux pump. These ABC transporter proteins include MDR1/P-glycoprotein (P-gp),[3] a number of the multidrug resistance-associated protein (MRP) family,[4] and the recently identified breast cancer resistance protein (BCRP).[5] The lung resistance-related vault protein (LRP) has been identified as the major vault protein (MVP), which is also associated with MDR.[6]

Nuclear expression of YB-1 has been reported to have a close relationship with MDR1/P-gp expression,[7–9] or poor prognosis in several human malignancies.[7,9] YB-1 also has been reported to promote basal and 5-fluorouracil-induced expression of the *MVP* gene, the promoter of which contains the Y-box in human colon cancer.[10] Moreover, nuclear expression of YB-1 has demonstrated significant correlation with intrinsic MVP expression in ovarian cancer.[11]

Rhabdomyosarcoma (RMS) is the most common malignant soft tissue neoplasm in children.[12] Based on histopathological features, RMS can be categorized into two major types: embryonal and alveolar subtype.[13] Alveolar RMS (ARMS) emerges as morphologically, genetically and biologically distinct from embryonal RMS (ERMS).[14–16] ARMS harbors non-random chromosomal translocations t(2;13)(q35;q14) or t(1;13)(p36;q14) that lead to the fusion of *PAX3* and *PAX7*, respectively, to *FKHR*.[17,18] In ERMS no diagnostic specific genetic alterations have been demonstrated, however, molecular analysis of polymorphic loci revealed allelic loss in chromosomal region 11p15 in most cases.[19,20] RMS is now commonly treated using chemotherapeutic agents,[21,22] including vinca alkaloids, anthracyclines, etoposide, cyclophosphamide, and ifosfamide, which are involved in the substrates of the above ABC transporters,[3–5] or MVP.[6]

In this study, we analyzed the correlation between YB-1 nuclear expression and the intrinsic ABC transporter or MVP expression in 63 cases of RMS and the results of these expression patterns were compared between ARMS and ERMS. Furthermore, we compared intrinsic ABC transporter or *MVP* mRNA expressions between 18 cases of RMS and six samples of normal skeletal muscle, using the real-time quantitative reverse transcriptase (RT)-PCR method.

Materials and Methods

Case materials. Sixty-three cases of primary rhabdomyosarcoma registered in the soft-tissue sarcoma files at the Department of Anatomic Pathology, Kyushu University between 1971 and 2004 were available for immunohistochemical study. The diagnosis of all cases was based on light microscopic examination with hematoxylin-eosin staining according to the World Health Organization classification in 2002,[13] and, where necessary, immunohistochemical analysis was carried out

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including myogenin and/or MyoD1 expression to confirm skeletal muscle differentiation. All the samples were collected prior to the administration of any therapy. Among them, frozen material was available in 18 cases for the evaluation of mRNA expression. In all seven cases of ARMS where frozen material was available, PAX3-FKHR transcripts was detected by RT-PCR. Furthermore, six frozen samples of normal skeletal muscle from patients with other diseases were used as a non-tumorous control. Survival data were available for 61 cases. Follow up ranged from 2 to 223 months (mean, 47 months). Patients were considered as adults when diagnosed at 16 years or older.[23] The sites of the primary tumor were divided into favorable site (orbit, head and neck [excluding parameningeal]) and genitourinary system (non-bladder/non-prostate) and unfavorable site (bladder/prostate, extremity, parameningeal head and neck, and other sites [including trunk, retroperitoneum etc.]). Forty-two patients were treated with chemotherapy and surgery, eight were treated with surgery and irradiation, and six were treated with surgery, chemotherapy and irradiation. Three patients were treated with surgery alone, but details of the therapy were unknown in four patients. Before the treatment, the disease was classified into four stages according to the TNM staging system developed by the Intergroup Rhabdomyosarcoma Study (IRS).[24]

Immunohistochemistry. Formalin-fixed, paraffin-embedded blocks containing the most viable parts of the tumor were selected in each case. The following monoclonal antibodies were used as primary antibodies: anti-P-gp (JSB-1; 1:20; Sanbio, Uden, Netherlands), anti-MRP1 (MRPr1; 1:50; Nichirei, Tokyo, Japan), anti-MRP2/cMOAT (M_2III-6; 1:20; Sanbio), anti-MRP3 (M3 III-6, 1:80; Kamiya Biomedical Company, Seattle, WA, USA), anti-MVP (LRP56, 1:50; Nichirei), anti-BCRP (BXP-21, 1:50; CHEMICON, Temecula, CA, USA) and anti-Ki-67 (MIB-1; 1:100; Dako Cytomation, Glostrup, Denmark). The antibody to YB-1 was anti-YBC polyclonal antibody prepared against a 15-amino acid synthetic peptide in the COOH-terminal domain.[25] This antibody was used at a working dilution of 1:100.[8] Four micrometer-thick sections were stained using a streptavidin-biotin-peroxidase method (HISTOFINE SAB-PO kit, Nichirei). For staining in the cases of all the antibodies, sections were pretreated with microwave irradiation for the purpose of antigen retrieval.

MDR human osteosarcoma cell line MNNG/HOS/DXR 1000,[26] served as a control for JSB-1, while tissue from the adrenal gland served as a control for MRPr1. Tissue from a normal liver and colon was used as a control for MRP2/cMOAT and MRP3, respectively. Moreover, tissue from normal kidney,[27] and placenta,[5] served as a control for MVP and BCRP, respectively. For each procedure, a negative control was also obtained by staining the samples with secondary antibody only.

ABC transporter proteins or LRP-positive tumor samples were graded from 0 to 3 according to the distribution of positivity and the degree of immunostaining of the plasma membrane or cytoplasmic Golgi region,[28,29] as follows: Score 0, no immunoreactive tumor cells were detected; Score 1, less than 10% of the tumor cells are positive, with weak immunostaining; Score 2, more than 10% of the tumor cells are positive, with weak immunostatining; Score 3, more than 10% of the tumor cells are positive, with strong immunoreactivity. The highest degree of positivity found in any area of the section was recorded,[28,30] and a score of 2 or 3 was judged as high expression. The immunoreactivity in each case was judged independently by three pathologists (YO, HY, ST). The MIB-1-labeling index (LI) was estimated by counting the number of positive cells per 1000 tumor cells.

Real-time quantitative RT-PCR. Total RNA was extracted using Trizol Reagent (Invitrogen Corp., Carlsbad, CA, USA) and reverse transcription was performed with Superscript II reverse transcriptase (Invtrogen Corp.) according to the manufacturer's instructions. Real-time quantitative PCR (TaqMan PCR) using an ABI PRISM 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) was performed according to the manufacturer's protocol. The sequences of oligonucleotide primer pairs and TaqMan probes for, *MRP1*, *MRP2* and *MRP3* are as previously described.[30]

Real-time quantitative PCR for *MDR1*, *MVP* and *BCRP* was performed using predeveloped TaqMan assay reagents of human *MDR1 (ABCB1)* (spanning exon 6/exon 7; ID: Hs00184500-m1), *MVP* (spanning exon 2/exon 3; ID: Hs00233856-m1), and *BCRP (ABCG2)* (spanning exon 5/exon 6; ID: Hs00184979-m1). Primers and probes for *GAPDH* were purchased from Perkin-Elmer Applied Biosystems (TaqMan GAPDH control reagent kit). All the reactions for standard samples and samples of patients were performed in triplicate. The data were averaged from the values obtained in each reaction. The mRNA levels of each of the genes were standardized by *GAPDH* and estimated as previously described.[30]

Statistical analysis. The difference in mRNA expression between tumor tissue and skeletal muscle as a control, and the correlation between real-time quantitative RT-PCR and immunohistochemistry were evaluated by the Mann–Whitney U and Kruskal–Wallis test. Association between two variables was evaluated by a two-sided chi-square test. The difference in MIB-1-LI between the two groups was estimated by unpooled two-sample *t*-test. The outcome of differences in various factors was compared by the log-rank test. Multivariate survival analysis was performed with a Cox proportional hazards regression model. A P of less than 0.05 was considered statistically significant.

Results

Patient characteristics. The clinical and pathological data for the patients with RMS are summarized in Table 1. There were

Table 1. Clinical characteristics of 35 ERMS and 28 ARMS patients

Characteristics	ERMS	ARMS
Age		
<16 years (n = 41)	27	14
≥16 years (n = 22)	8	14
Gender		
Male (n = 31)	16	15
Female (n = 32)	19	13
Anatomic site of the primary tumor		
Favorable site: (n = 16)	11	5
Orbit	0	1
Head and neck (excluding parameningeal)	3	4
GU-Nonbladder/Nonprostate	8	0
Unfavorable site: (n = 47)	25	22
Bladder/Prostate	7	0
Extremity	7	10
Head and neck, paramenigeal	2	6
Other (trunk, retroperitoneum, etc.)	9	6
Tumor size		
<5 cm (n = 21)	11	10
≥5 cm (n = 37)	23	14
Unknown (n = 5)	1	4
IRS Stage		
Stage 1 (n = 14)	9	5
Stage 2 (n = 5)	4	1
Stage 3 (n = 37)	21	16
Stage 4 (n = 2)	0	2
Unknown (n = 5)	1	4

GU, genitourinary system; IRS, Intergroup Rhabdomyosarcoma Study. ERMS, embryonal rhabdomyosarcoma; ARMS, alveolar rhabdomyosarcoma.

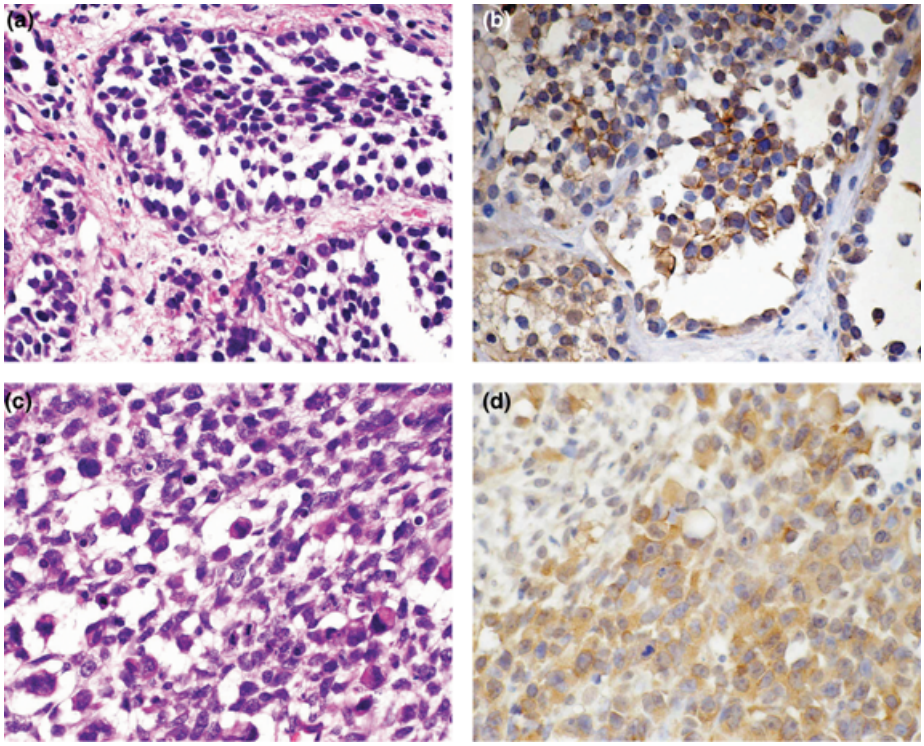

Fig. 1. Immunohistochemical expression of ATP-binding cassette transporters in rhabdomyosarcoma. Alveolar rhabdomyosarcoma arising in the nasal cavity of a 28-year-old man (a) shows strong membranous immunoreaction for P-glycoprotein (b; Score 3). Embryonal rhabdomyosarcoma in the retroperitoneum of a 4-year-old girl (c) shows both membranous and cytoplasmic localization of breast-cancer resistant protein (d; Score 3).

31 male and 32 female patients, ranging in age from 15 days to 39 years (mean, 11.0 years). Forty-one patients were children, while 22 patients were adults. Histologically, 35 tumors were classified as embryonal type, including three botryoid type and four spindle cell type. Twenty-eight tumors were categorized as alveolar type.

Immunohistochemical expression of ABC transporters, LRP and the expression of their mRNAs. Immunohistochemically, high expression of P-gp was observed in 32 out of 63 cases (51%, Fig. 1a,b). High expression of MRP1 protein was identified in 33 out of 63 cases (52%), whereas high MRP2/cMOAT expression was found in 22 cases (35%). Twenty-five tumors showed high MRP3 protein expression (40%). High expression of MVP and BCRP (Fig. 1c,d) protein was recognized in 30 (48%) and 22 (35%) cases, respectively. Concerning histological subtype, ARMS showed significantly more frequent P-gp expression (Fig. 1a,b), compared with ERMS (Table 2, $P = 0.0332$), whereas the expression of BCRP was significantly higher in ERMS (Fig. 1c,d) in comparison with that in ARMS (Table 2, $P = 0.0184$).

Tumor tissue expressed *MDR1* mRNA at a significantly higher level (57.12 arbitrary units [A.U.], mean) than the control skeletal muscle tissue (0.31 A.U., mean) ($P = 0.0429$). The levels of *MRP1* (mean, 33.47 A.U.), *MRP3* (mean, 6210.6 A.U.) and *LRP* (mean, 980.13 A.U.) mRNA expression in tumor tissue were also significantly higher than those in the control tissue (*MRP1*: mean, 0.85 A.U., $P = 0.0234$, *MRP3*: mean, 0.54 A.U., $P = 0.0004$, *LRP*: mean, 0.09 A.U., $P = 0.0003$). Concerning *MRP2* and *BCRP* mRNA expression, no statistically significant difference was recognized between tumor tissue and the control tissue. When the results from immunohistochemistry and real-time quantitative RT-PCR techniques were compared, a statistical association was found between immunoreactivities and mRNA expression levels for all ABC transporters and *LRP* (Table 3 and Fig. 2).

YB-1 protein expression. All 63 cases of RMS showed positive immunoreaction for anti-YBC in the cytoplasm with uniform intensity (Fig. 3a). In 24 of the 63 cases (38%), YB-1 expression was also recognized in the nucleus (Fig. 3b). YB-1 nuclear

Table 2. Comparison of immunohistochemical results between embryonal and alveolar rhabdomyosarcoma

Protein		ERMS ($n = 35$)	ARMS ($n = 28$)	*P*-value
YB-1	Nuclear	12	12	$P = 0.3313$
	Cytoplasm	23	16	
P-gp	Score 0	10	3	$P = 0.0332$*
	1	13	5	
	2	6	9	
	3	6	11	
MRP1	Score 0	14	4	$P = 0.0713$
	1	4	8	
	2	9	6	
	3	8	10	
MRP2	Score 0	8	9	$P = 0.1761$
	1	16	8	
	2	4	8	
	3	7	3	
MRP3	Score 0	10	7	$P = 0.3645$
	1	13	8	
	2	9	6	
	3	3	7	
MVP	Score 0	11	6	$P = 0.4957$
	1	10	6	
	2	8	7	
	3	6	9	
BCRP	Score 0	5	14	$P = 0.0184$*
	1	14	8	
	2	6	3	
	3	10	3	
MIB-1-LI		18.26 ± 13.59	12.23 ± 7.71	$P = 0.0407$*

*Statistically significant.
ARMS, alveolar rhabdomyosarcoma; BCRP, breast-cancer resistant protein; ERMS, embryonal rhabdomyosarcoma; MIB-1-LI, MIB-1-labeling index; MRP, multidrug resistance-associated protein; MVP, major vault protein; P-gp, P-glycoprotein; YB-1, Y-box-binding protein-1.

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Table 3. Correlation between protein and mRNA expression

Protein		mRNA (A.U.) mean	SD	MW-test**	KW-test***
P-gp	Score 0 or 1 (n = 9)	0.579	0.909	P = 0.0003*	P = 0.0037*
	Score 2 or 3 (n = 9)	113.656	156.308		
MRP1	Score 0 or 1 (n = 5)	1.422	1.402	P = 0.0137*	P = 0.0077*
	Score 2 or 3 (n = 13)	45.79	52.754		
MRP2	Score 0 or 1 (n = 9)	0.67	1.262	P = 0.0152*	P = 0.0222*
	Score 2 or 3 (n = 9)	8.532	14.628		
MRP3	Score 0 or 1 (n = 6)	208.669	301.488	P = 0.0159*	P = 0.0057*
	Score 2 or 3 (n = 11)	9484.374	21 576.062		
MVP	Score 0 or 1 (n = 10)	112.14	239.27	P = 0.033*	P = 0.0075*
	Score 2 or 3 (n = 8)	2065.115	3995.216		
BCRP	Score 0 or 1 (n = 7)	0.113	0.157	P = 0.0063*	P = 0.0142*
	Score 2 or 3 (n = 11)	32.538	50.236		

*Statistically significant, **Mann–Whitney (MW) *U*-test (comparison of two groups/Score 0 or 1 *versus* Score 2 or 3); ***Kruskal–Wallis (KW) test in four groups (Score 0, 1, 2, 3). A.U., arbitrary units; BCRP, breast-cancer resistant protein; MRP, multidrug resistance-associated protein; MVP, major vault protein; P-gp, P-glycoprotein; SD, standard deviation.

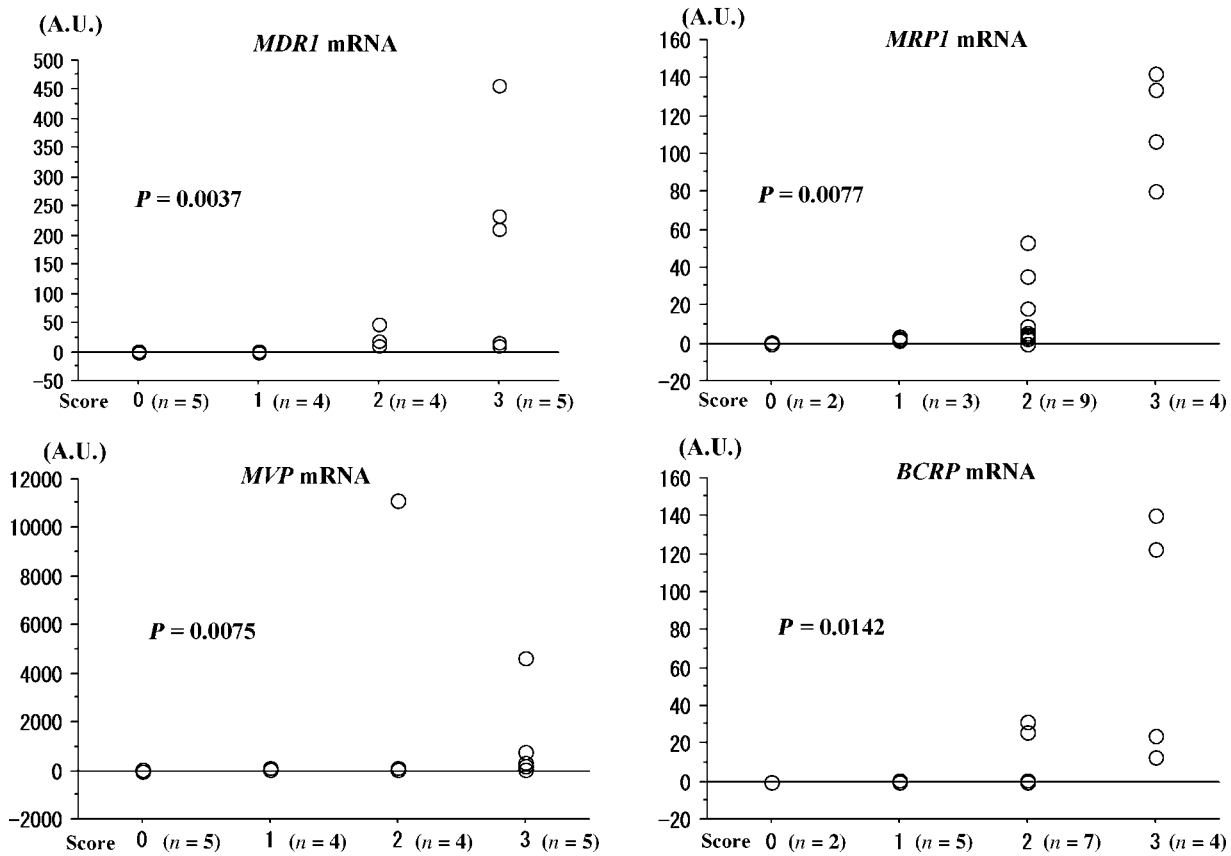

Fig. 2. Correlation between mRNA and immunohistochemical expression status of P-glycoprotein, multidrug resistance-associated protein (MRP)1, major vault protein (MVP) and breast-cancer resistant protein (BCRP). The immunohistochemical status was significantly correlated with the concordant mRNA expression ($P < 0.05$, Kruskal–Wallis test). A.U., arbitrary units.

expression was observed in 12 of 35 ERMS (34%), whereas it was recognized in 12 of 28 ARMS (43%). In ERMS, significant correlation was also recognized between YB-1 nuclear expression and P-gp ($P = 0.0359$) or MVP ($P = 0.0044$) (Table 4). On the other hand, in ARMS no such relationship was observed.

MIB-1-li. In ERMS, the cases with YB-1 nuclear expression also showed a significantly higher MIB-1-LI (mean, 25.299), compared with the cases with cytoplasmic YB-1 expression (mean, 14.584) (Table 4, $P = 0.0244$). However, no such difference was observed in ARMS. Moreover, the MIB-1-LI of the cases with high BCRP expression was significantly higher (mean, 19.88) than that of the cases without high BCRP expression (mean, 13.27) ($P = 0.0311$).

Survival analysis. The results of survival analysis are summarized in Table 5. Adult patients showed significantly poor prognosis, compared with children ($P = 0.0125$). The cases with an unfavorable site ($P = 0.0739$), a high IRS stage (stage 3 or 4) ($P = 0.0843$) showed poor survival by univariate analysis, however, these results showed no statistical significance. On the

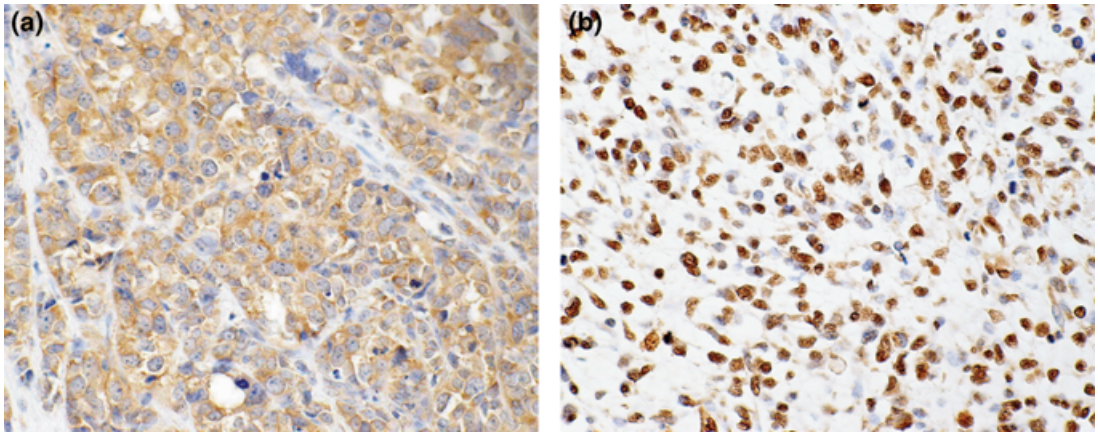

Fig. 3. (a) Alveolar rhabdomyosarcoma arising in the vulva of a 21-year-old woman. Y-box-binding protein-1 (YB-1) expression is observed only in the cytoplasm. This tumor showed negative immunoreactivity for P-glycoprotein (P-gp) (Score 0). (b) Embryonal rhabdomyosarcoma arising in the testis of a 14-year-old boy. Diffuse and strong nuclear expression of YB-1 protein is evident in the tumor cells. This tumor showed high expression of P-gp (Score 3).

other hand, multivariate analysis including clinicopathological and immunohistochemical parameters revealed age at diagnosis (≥16 years old: $P = 0.0072$), MRP1 ($P = 0.0244$) and MRP2 ($P = 0.0326$) expressions were an independent and significant factors for poor prognosis.

Table 4. Correlation between YB-1 nuclear expression and P-glycoprotein or MVP expression, and MIB-1-LI

		Embryonal RMS		
	YB-1	Nuclear	Cytoplasm	
P-gp	Score 0	2	8	$P = 0.0359$*
	1	2	11	
	2	4	2	
	3	4	2	
MVP	Score 0	0	11	$P = 0.0044$*
	1	3	7	
	2	4	4	
	3	5	1	
		Alveolar RMS		
	YB-1	Nuclear	Cytoplasm	
P-gp	Score 0	1	2	$P = 0.9827$
	1	2	3	
	2	4	5	
	3	5	6	
MVP	Score 0	3	3	$P = 0.494$
	1	1	5	
	2	3	4	
	3	5	4	
		Embryonal RMS		
	MIB-1-LI	Mean	SD	
YB-1	N ($n = 12$)	25.299	15.298	$P = 0.0244$*
	C ($n = 23$)	14.584	11.274	
		Alveolar RMS		
	MIB-1-LI	Mean	SD	
YB-1	N ($n = 12$)	14.501	6.278	$P = 0.1822$
	C ($n = 16$)	10.528	8.424	

*Statistically significant. C, cytoplasmic; MIB-1-LI, MIB-1-labeling index; MVP, major vault protein; P-gp, P-glycoprotein; N, nuclear; RMS, rhabdomyosarcoma; SD, standard deviation; YB-1, Y-box-binding protein-1.

Discussion

There have been some investigations concerning the expression of MDR1/P-gp or MRP1 in a large series of RMS patients.[23,31–33] Gallego *et al*.[32] demonstrated frequent *MDR1* and *MRP1* mRNA expression in ARMS compared with ERMS, whereas other studies,[23,31] have failed to reveal any differences in P-gp or MRP1 protein expression between ARMS and ERMS. In the current study, ARMS showed significantly frequent P-gp expression in comparison with ERMS. Frequent chemoresistant phenotype in ARMS may be due to frequent P-gp expression. Moreover, in the current study, MRP1 expression was one of the adverse prognostic factors, using multivariate analysis.

As for MVP expression in RMSs, Komdeuer *et al*.[23] reported either no expression or only limited expression of MVP in ARMS compared with ERMS. Moreover, their group also demonstrated that MVP expression was most prominent in the more differentiated tumor cells in untreated tumors and that its expression increased significantly following chemotherapy.[33] Accordingly, they concluded that MVP plays some part in therapy-induced differentiation. In our series, MVP expression was recognized in both undifferentiated and differentiated tumor cells, and no difference was observed between ERMS and ARMS with regard to MVP expression.

BCRP (ABCG2) also belongs to the ABC transporter family and its sequence is similar to one-half of the duplicated P-gp or MRP1 molecule.[5] BCRP affects a narrow range of anticancer agents compared to the MDR1 (ABCB1) and MRP (ABCC) transporters, including anthracyclines, mitoxantrone and topoisomerase I inhibitors.[34] Although a close correlation between the overexpression of BCRP and poor prognosis has been reported in adult acute myeloid leukemia,[35] there have been no investigations of BCRP expression in malignant pediatric solid tumors, including RMS. In this study, BCRP expression was significantly more frequently observed in ERMS compared with ARMS. Moreover, the cases with high BCRP expression showed higher proliferating activities as measured by the MIB-1-LI.

Previous studies have demonstrated a close relationship between the nuclear expression of YB-1 and MDR1/P-gp expression in several kinds of human malignancies.[7–9] We previously demonstrated that YB-1 nuclear expression also had a significant correlation with high proliferative activities as determined by the MIB-1-LI in human osteosarcoma.[8] Stein *et al*.[10] demonstrated an increased expression of endogenous MVP protein by transduction of YB-1 cDNA *in vivo*, and a strong correlation between MVP and YB-1 expression in human colon

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Table 5. Overall survival in 61 cases of rhabdomyosarcoma

Variable		*P*-value in survival analysis	
		Univariate	Multivariate
Clinicopathologic			
Age	<16 years (*n* = 39) ≥16 years (*n* = 22)	0.0065*	0.0072*
Sex	Male (*n* = 30) Female (*n* = 31)	0.8254	0.0979
Site	Favorable (*n* = 15) Unfavoable (*n* = 46)	0.0755	0.4612
Histology	Embryonal (*n* = 33) Alveolar (*n* = 28)	0.2896	0.6479
Size	<5 cm (*n* = 20) ≥5 cm (*n* = 36)	0.214	0.0662
Stage	Low (1,2) (*n* = 18) High (3,4) (*n* = 38)	0.0914	0.3853
Immunohistochemical			
	YB-1 nuclear expression (–) (*n* = 37) (+) (*n* = 24)	0.4431	0.0538
	P-gp (Score 0 or 1) (*n* = 30) (Score 2 or 3) (*n* = 31)	0.1339	0.0948
	MRP1 (Score 0 or 1) (*n* = 30) (Score 2 or 3) (*n* = 31)	0.1397	0.0244*
	MRP2 (Score 0 or 1) (*n* = 39) (Score 2 or 3) (*n* = 22)	0.775	0.0326*
	MRP3 (Score 0 or 1) (*n* = 37) (Score 2 or 3) (*n* = 24)	0.707	0.298
	MVP (Score 0 or 1) (*n* = 32) (Score 2 or 3) (*n* = 29)	0.3501	0.1958
	BCRP (Score 0 or 1) (*n* = 41) (Score 2 or 3) (*n* = 20)	0.4945	0.4907
	MIB-1 LI (<15.7, mean) (*n* = 36) (≥15.7, mean) (*n* = 25)	0.1535	0.1169

*Statistically significant. BCRP, breast-cancer resistant protein; MIB-1-LI, MIB-1-labeling index; MRP, multidrug resistance-associated protein; MVP, major vault protein; P-gp, P-glycoprotein; YB-1, Y-box-binding protein-1.

cancer specimens. Moreover, we recently reported a close relationship between YB-1 nuclear expression and MVP expression in human ovarian cancer.[11] In the current study, significant relationships between YB-1 nuclear expression and P-gp/MVP expression, or a high MIB-1-LI were observed in only ERMS, with no such correlation being recognized in ARMS. On the other hand, P-gp expression was more frequently observed in ARMS compared with ERMS. Therefore, in ARMS, mechanism behind the up-regulation of P-gp may not be due to the YB-1 pathway and it may be quite different from that in ERMS. These different expression profiles of MDR-related proteins and YB-1 nuclear expression support the concept that ARMS is a distinct tumor which is molecular genetically different from ERMS,[16] except with regard to skeletal muscle differentiation which is manifest in both tumors.

The nuclear expression of YB-1 is reported to be associated with poor prognosis in several kinds of malignant solid tumors.[1,2,11] There have been no investigations concerning YB-1 nuclear expression in pediatric malignant solid tumors. In this study, YB-1 nuclear expression did not show any prognostic significance. Moreover, we could not find correlation between histological type, site and size with survival. These factors have been reported as predictive prognostic factors in RMS.[13] These discrepancies may be due to the fact the patients in this series were in heterogeneously treated groups. To elucidate the correlation between YB-1 nuclear expression and prognosis in RMS, further large studies in uniformly treated groups are needed.

In conclusion, ARMS and ERMS showed different expression profiles of MDR-related molecules and this result supports the theory that both tumors are molecular genetically distinct. YB-1 could be novel candidate for a therapeutic target, especially in cases of ERMS, because of the close correlation between YB-1 nuclear expression and P-gp in this tumor.

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Disclosure/conflict of interest

The authors declare no disclosures or conflicts of interest.

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