Modulation of HLA class I expression in multidrug-resistant human rhabdomyosarcoma cells*

C. Melguizo[1], J. Prados[1], J. A. Marchal[2], C. Vélez[1], E. Carrillo[2], H. Boulaiz[3], I. Sánchez-Montesinos[3], R. Madeddu[4], A. Aránega[3**]

[1]Department of Health Sciences, University of Almería, Almería E-04002, Spain; [2]Department of Health Sciences, University of Jaén, Jaén E-23071, Spain; [3]Department of Human Anatomy and Embriology, School of Medicine, University of Granada, Granada E-18071, Spain, e-mail: hboulaiz2@ugr.es; [4]Department of Biomedical Science, University of Sassary, Sassary 07100, Italy

Received October 30, 2002

An abnormal HLA expression has been detected in some tumors including rhabdomyosarcoma (RMS). Classical cytotoxic treatment of these tumors, the most common childhood soft tissue malignacy, may induce multidrug resistance (MDR) associated with the expression of a 170-kDa membrane-associated glycoprotein (P-glycoprotein). In order to analyse the connection between modulation of HLA expression and the development of the MDR phenotype mediated by P-glycoprotein in RMS, we used three resistant RMS cell lines; two of these resistant cell lines (TE.32.7.DAC and RD-DAC) were established by *in vitro* exposure to actinomycin D, a drug of choise in the treatment of RMS; the resistant RMS-GR cell line was established from an embryonal RMS tumor after polychemotherapy. Our results showed that all the resistant cell lines showed a significant increase in the expression of HLA class I surface antigens in comparison to drug-sensitive cells. Blockade of P-glycoprotein with verapamil led to a decrease in HLA class I expression in RMS resistant cell lines. However, no modulation of HLA class II expression was observed in any of the three analyzed cell lines. These findings support the hypothesis that the development of resistance mediated by *mdr 1*/P-glycoprotein, directly influences the expression of HLA class I in RMS cells, inducing to upregulation. This effect may be relevant to the application in RMS of immunotherapy against tumor-associated antigens presented by HLA class I molecules.

Key words: MDR, P-glycoprotein, rhabdomyosarcoma, HLA, verapamil.

The development of multidrug resistance (MDR) remains the major limitation in the chemotherapy of malignancies [33], as show the large amount of strategies which have been developed to modulate this phenomenon [30]. Although several mechanisms have been described by which tumor cells may express drug resistance, classical MDR has been associated with the expression of a 170-kDa membrane-associated glycoprotein (P-glycoprotein) that serves as a drug efflux pump [17]. Rhabdomyosarcomas (RMS), the most common childhood soft tissue malignacy [26], are characterized by poor response to cytotoxic treatment and significant morbidity [2, 9]. Clinical [24] and experimental [7, 25] studies showed an increase in *mdr 1*/P-glycoprotein expression after chemotherapy, suggesting that this resistance mechanism may explain the frequent failure of cytotoxic therapy in RMS. Recently, it has been showed that the development of this resistance may be preventable in RMS by using modulators of MDR [6].

Modulation of HLA expression has been detected during malignant transformation. The clinical significance of this change in tumor cells is not clear, although it has been related with the degree of differentiation and prognosis, metastatic potential [1] and with the development of the MDR phenotype mediated by P-glycoprotein [34]. This member of the ABC superfamily is able to transport across plasma

*This study was supported by the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III, through project 01/1092.

**Author to whom correspondence should be sent.

membranes short peptides which are necessary for the correct folding, transport and cell membrane expression of MHC class I proteins [16, 31]. Interestingly, *in vivo* studies showed elevated HLA class I expression in a high proportion of RMS [10], although no data have been published about alterations in HLA expression related with the development of MDR in muscular neoplasms.

In this study we analyze the correlation between HLA and P-glycoprotein expression in three human RMS cell lines characterized by a MDR phenotype. The findings may be relevant to understand the immune response againts RMS.

Material and methods

Cell lines. We used three different resistant RMS cell lines obtained by different methods. Two of these cell lines, the resistant TE.32.7.DAC [25] and RD-DAC [21] were established from the TE.32.7 and RD human embryonal RMS cell lines by exposure to increasing concentrations of actinomycin D in the culture medium. Both parental cell lines were obtained from the American Type Culture Collection (ATCC). The resistant RMS-GR cell line [11] was established from an embryonal RMS tumor biopsied in a patient who had been treated with chemotherapy (doxorubicin, vincristine, cyclophosphamide and dactinomycin). All cell lines were grown at 37 ˚C in an atmosphere containing 5% CO_2, with Dulbecco's modified Eagle medium (MEM) (Gibco, Grand Island, NY) supplemented with 10% FBS (Gibco), 20 mM L-glutamine, 3.5 mg/μl sodium bicarbonate, 4.5 g/l glucose, 250 U/ml ampicillin and 20 μg/ml streptomycin.

Northern blotting. Cells were trypsinized at the end of the exponential phase of growth, centrifuged and stored in liquid nitrogen. Total RNA was obtained from frozen cells according to Sambrook et al [27]. Twenty micrograms of RNA were fractionated on agarose gels (1%) in the presence of 18% formaldehyde (vol/vol). Northern blot hybridization [12] was performed using nylon membranes and a ^{32}P-label oligoprobe recognizing *mdr 1* sequences (cDNA sequence 3027-3049) (106 dpm/ml). The exposure time of the autoradiograms was 24–120 h. To demonstrate the integrity of the RNA preparations hybridization was performed using a β-actin oligoprobe (cDNA sequence 1874–1898).

Preparation of RMS cells for FACScan. Briefly, 10^6 cells were transferred to universal screw cap tubes containing phosphate-buffered saline (PBS), then washed and centrifuged at 225 g for 5 min. The supernatant was discarded, and the washing and centrifugation steps were repeated twice. To determine HLA class I and class II expression, the cells were fixed with 2% formaldehyde for 10 min at –20 ˚C and immediately washed three times in PBS at 4 ˚C. The cells were incubated for 30 min at 4 ˚C with the monoclonal antibodies (mAbs) W6/32 and GRH1 against a common HLA class I (A, B, C heavy chain) determinant and β_2-microglobulin, respectively [4, 19] and with the GRB1 mAb against HLA class II molecules [5], then washed twice with cold PBS and reincubated with fluorescein isothiocyanate-conjugated antimouse immunoglobulin (Sigma, St. Louis, MO) (1:50) for 30 min at 37 ˚C. To determine P-glycoprotein expression the cells were permeabilized with Triton X-100 (0.05%) at room temperature for 10 min and incubated with the mAb JSB-1 (5 μl) [28]. The rest of the procedure was done as described above. The expression of P-glycoprotein was assessed using the mAb C-219 according to the basic protocol recommended by the manufacturer (Centacor, Inc; Malvern, PA). The results were obtained as mean fluorescence. The percent increase in mean fluorescence was calculated with the formula: (MFI-MFB/MFB)x100, where MFI is the mean induced fluorescence and MFB is the mean basal fluorescence.

Cytotoxicity experiments in RMS cell lines. To evaluate P-glycoprotein blockade, resistant and sensitive RMS cells ($2x10^5$/ml) were treated with different concentrations of actinomycin D, vincristine or doxorubicin in four replicate samples in the absence or presence of verapamil at a non-toxic concentrations (10 μM) for 1 h. After 72 h, cells were harvested by trypsinization and counted in a model ZBI Coulter Counter (Hialeah, FL). Cell viability was determined with trypan blue dye exclusion. The dose that inhibed 50% of growth (ID_{50}) was calculated from the curve for the percentage of cell survival at different drug concentrations.

Statistical analysis. All data are expressed as the mean $\pm$SEM. The Student's t test was used to determine the level of significance. Difference were considered significant at $p<0.05$.

Results

Mdr1/P-glycoprotein expression in RMS cells lines. As shown in Figure 1, TE.32.7.DAC, RD-DAC and RMS-GR cell lines showed a positive mdr1 expression. Analysis of the Northern blot bands (normalized by comparisons with the β-actin signal of each sample) showed a slightly larger mRNA mdr1 level in RMS-GR that in RD-DAC and TE.32.7.DAC resistant cell lines. FACScan analyses of these resistant RMS cell lines with J-SB1 showed that the MDR phenotype was characterized by elevated levels of P-glycoprotein expression. C-219 staining confirmed the high expression of P-glycoprotein in the three cell lines (Fig. 2).

HLA class I and II cell surface expression in RMS cell lines showing an MDR phenotype. The W6/32 mAb staining showed a significant increase in HLA class I expression in RMS resistant cells, when compared to the parental drug-

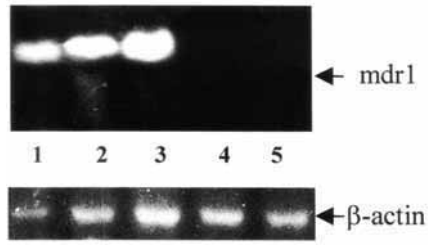

Figure 1. Northern blot analysis of mdr1 expression in RMS cells. β-actin hybridization was realized to demonstrate the integrity of the RNA preparations. 1 – RD-DAC, 2 – TE-32.7-DAC, 3 – RMS-GR, 4 – TE-32.7, 5 – RD.

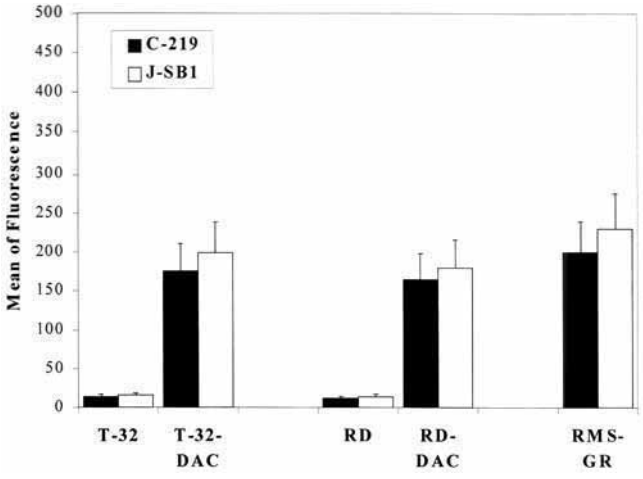

Figure 2. Analysis by FACScan of P-Glycoprotein expression using J-SB1 and C-219 mAbs in RMS cells. Results are expressed as mean of fluorescence and are representative for four independent experiments.

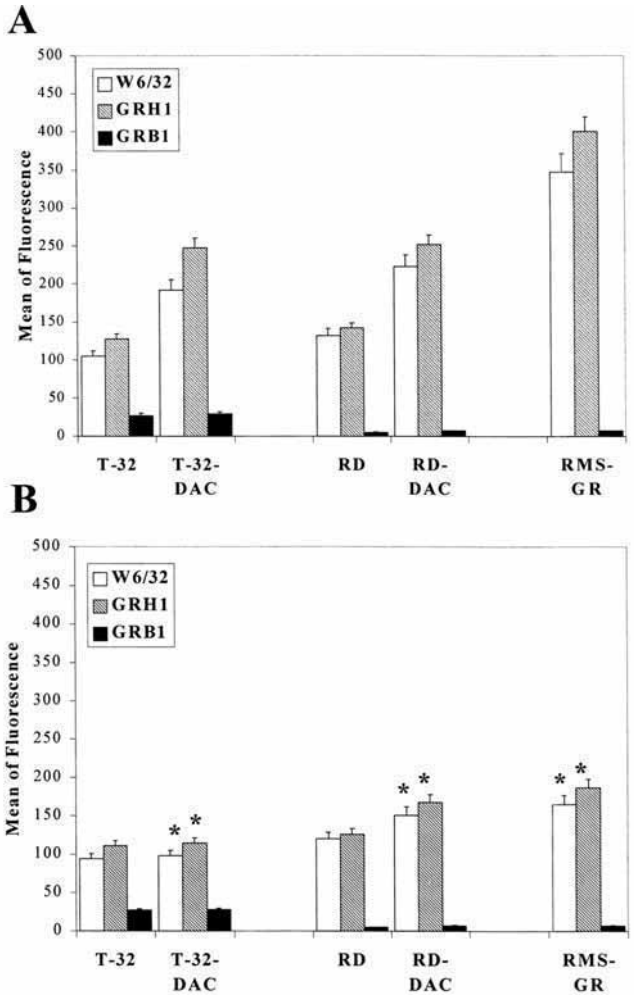

Figure 3. Analysis by FACScan of HLA class I (W6/32 and GRH1 mAbs) and HLA class II (GRB1 mAb) antigens expression in RMS cell lines before (A) and after exposure of cells to verapamil at a nontoxic concentrations (10 μM) for 1 h (B). Results are expressed as mean fluorescence and are representative of four independent experiments. Significance of the differences was determined by statistical comparison of the means between RMS cells before (A) and after (B) verapamil treatment. Values vere considered significant at $^{*}p<0.05$.

sensitive cells. Cell line TE.32.7-DAC showed an 82% increase in mean fluorescence in relation to the parental cell line. This increase was slightly greater than that found in RD-DAC (68.9%) (Fig. 3A). Analyses with the GRH1 mAb confirmed the increase in HLA class I expression in both TE.32.7-DAC and RD-DAC resistant cell lines (93.7% and 77.4% increase in mean fluorescence, respectively) (Fig. 3A). In contrast, HLA class II antigen expression detected with the specific GRB1 mAb was low in parental drug-sensitive cell lines and was not significantly modified in these resistant cell lines (Fig. 3A). The RMS-GR cell line, established from a primary tumor after polychemotherapy, showed a similar behaviour as the resistant cell lines obtained by exposure to increasing concentrations of actinomycin D. The analyses with both W6/32 and GRH1 mAbs, showed high levels of HLA class I expression in this resistant cell line, while the GRB1 mAb showed a weak HLA class II antigen expression (Fig. 3A).

Effect of pharmacologic blockade of P-glycoprotein in RMS cells. In order to determine the degree of P-glycoprotein blockade with verapamil we evaluated the modification of actinomycin D, vincristine and doxorubicin cytotoxicity in parental and resistant RMS cell lines. An approximately 33-, 4- and 70-fold increase in the effect of actinomycin D, vincristine and doxorubicin, respectively, ocurred in TE.32.7-DAC cells with verapamil as compared to the ID_{50} values (Tab. 1). Verapamil at the same nontoxic concentration greatly enhanced the cytotoxicity of drugs in resistant RD-DAC cells in which the increase in the effect of actinomycin D, vincristine and doxorubicin was 17-, 5- and 40-fold, respectively. The same experience in RMS-GR showed that the increase in the effect of the three drugs was 53-, 77- and 9- respectively (Tab. 1). In contrast, slight modifications in drug cytotoxicity were found in drug-sensitive TE.32.7 and RD cell lines (Tab. 1).

Table 1. Resistant and sensitive RMS cells. Effect of verapamil treatment (10 μM) on the ID_{50} (nM).

Drugs	RD-DAC		RD		TE.32.7-DAC		TE.32.7		RMS-GR	
	A	B	A	B	A	B	A	B	A	B
Actinomycin	12.831 $\pm$0.37	0.742 $\pm$0.08* (17.2)	0.860 $\pm$0.32	0.614 $\pm$0.07 (1.4)	30.14 $\pm$0.42	0.9 $\pm$0.09* (33.4)	1.53 $\pm$0.15	0.8 $\pm$0.07 (1.9)	175 $\pm$13	3.3 $\pm$0.35* (53)
Vincristine	3.40 $\pm$0.7	0.653 $\pm$0.06* (5.2)	0.07 $\pm$0.0002	0.058 $\pm$0.05 (1.2)	7.45 $\pm$0.90	1.7 $\pm$0.08* (4.3)	0.12 $\pm$0.003	0.085 $\pm$0.02 (1.4)	5.8 $\pm$0.51	0.075 $\pm$0.01* (77)
Doxorubicin	106 $\pm$20	2.63 $\pm$0.13* (40.2)	2.92 $\pm$0.01	1.12 $\pm$0.09 (2.6)	162.4 $\pm$15	2.3 $\pm$0.026* (70.4)	4.01 $\pm$0.06	1.62 $\pm$0.21 (2.4)	17 $\pm$2.36	1.8 $\pm$0.17* (9)

The increase of drug cytotoxicity, indicated in parentheses, was calculated as the ratio between ID_{50} of the cell line in the absence (A) and presence (B) of verapamil. All data are means $\pm$SEM of four independent experiment. Significance was calculated by comparison of the ID_{50} values with Student's t test. Significance of the differences (*$p<0.05$) was determined by statistical comparison of the values in the absence (A) and presence (B) of verapamil.

Effect of the pharmacologic blockade of P-glycoprotein on the MHC Class I molecules in resistant cell lines. To verify that P-glycoprotein can affect HLA expression, we performed assays with verapamil. The ability of verapamil to modulate the cell surface level of the HLA class I was confirmed in sensitive and resistant cell lines. After treatment with verapamil we found a clear decrease in the expression of epitope recognized by W6/32 in resistant RMS cell lines obtained by exposure to actinomycin D. Fluorocytometric analyses showed a decrease of 49% and 32% in mean fluorescence intensity in TE.32.7-DAC and RD-DAC resistant cells, respectively (Fig. 3B). The decrease in the expression of epitope recognized by W6/32 was lower in both RD and TE.32.7 sensitive cell lines (Fig. 3B). Analyses using GRH1 mAb showed a decrease in the expression of β_2-microglobulin and confirmed the reduction observed in the expression of HLA class I with W6/32 mAb (54% and 33% decrease in mean fluorescence in TE.32.7-DAC and RD-DAC, respectively). In contrast, analyses of HLA class II expression using GRB1 mAb showed no modification in sensitive or resistant cells after treatment with verapamil (Fig. 3B). The W6/32 and GRH1 mAb staining also showed a significant decrease in HLA class I expression in the resistant RMS-GR cell line after treatment with verapamil (52% and 53%, respectively). However, no significant modulation of HLA class II expression was observed in this cell line (Fig. 3B).

Discussion

The coordinate activity of different transporters may be involved in the control of the MHC class I expression during the process of cellular differentiation [13]. The homology found between P-glycoprotein and the putative peptide transporters TAP1 and TAP2 suggests that this protein may act in accumulating short peptides into a vesicular export system [31]. Because the amount of the MHC class I on the cell surface is related to the quantity and quality of peptides in the endoplasmic reticulum [23], transport of protein fragments from the cytosol to the endoplasmic reticulum by P-glycoprotein may modulate HLA expression. Although studies in leukemic cells [34] and carcinoma cells [20] showed that *mdr 1* was not independent of HLA expression, the relationship of *mdr 1*/P-glycoprotein to HLA antigens in muscular neoplasms remains largely unclear. In order to verify the influence of *mdr 1* on HLA class I expression in resistant RMS cells, we used three resistant RMS cell lines and analyzed the effects of brief treatment with verapamil, which is able to block P-glycoprotein function [3]. Verapamil blocked P-glycoprotein increasing the cytotoxic effect in RMS-resistant cells and modulated HLA class I expression, indicating that, at least in the cell lines analyzed, the MDR phenotype was associated with a significant upregulation of MHC class I expression. This result was observed not only in the resistant cell lines obtained by *in vitro* exposure to actinomycin D but also in the cell line obtained from a patient treated with polychemotherapy. However, in contrast to the results obtained in HL-60/VC cell line [29], no modulation of HLA class II was observed in any of our RMS resistant cell lines.

Interestingly, modulation of HLA class I expression in tumor cells has been related to the susceptibility to inmunotherapy [15] and to the metastatic potential [1]. Firstly, the modulation of HLA in RMS cells which developed resistance against classical drugs used in their treatment may be relevant to the immunotherapy strategies based in tumor-associated antigens (such as MAGE, BAGE and GAGE) that are presented by these molecules [8]. On the other hand, metastatic potential is frequently increased in resistant tumors cells. The last point has been widely demonstrated in colon cancer [32], breast tumors [14], malig-

nant melanoma [18] and neuroblastoma [12]. The increase in HLA class I expression in resistant cells of glioblastoma multiforme [22] suggests the posibility that the metastatic potential of resistant tumor cells is related with the modulation in HLA, although the mechanism is unclear. In this context, the significant increase in HLA class I expression found in our resistant RMS cell lines, which was concordant with significant proportion of RMS that show elevated HLA class I antigen expression [10], raises the possibility that modifications in HLA class I expression in RMS resistant cells may be related to an increase in the metastatic potential of these cell lines. This hypothesis is now under investigation.

In conclusion, our data showed a correlation between the expression of *mdr1* and HLA class I in RMS cells. This finding suggests that the development of multidrug resistance in RMS cells may contribute to the regulation of the locoregional immune response of these tumors and may be important in their biological behavior. Further *in vivo* studies it will be necessary to determine the implications of modulation of HLA class I expression by the development of MDR mediated by mdr 1.

We thank Dr. F. Garrido of the Immunology Service Virgen de las Nieves Hospital in Granada for providing the monoclonal antibodies against HLA antigens, and Miss K. Shashok for improving the English style of the manuscript.

References

[1] Algarra I, Cabrera T, Garrido F. The HLA crossroad in tumor biology. Human Immunol 2000; 61: 65–73.

[2] Atra A, Pinkerton R. High-dose chemotherapy in soft tissue sarcoma in children. Crit Rev Oncol Hematol 2002; 41: 191–196.

[3] Avendano C, Menendez JC. Inhibitors of multidrug resistance to antitumor agents. Curr Med Chem 2002; 9: 159–193.

[4] Barnstable CJ, Bodmer WF, Brown G, Galfre G, Milstein C, Williams AF, Ziegler A. Production of monoclonal antibodies to group A erythrocytes, HLA and other human surface antigens: new tools for genetics analysis. Cell 1978; 14: 2–9.

[5] Cabrera T, Ruiz-Cabello F, López M, De La Higuera B, Sánchez M, Garrido F. Characterization of monoclonal antibodies against HLA class II molecules. Hybridoma 1986; 5: 191–198.

[6] Cocker HA, Tiffin N, Pritchard-Jones K, Pinkerton CR, Kelland LR. In vitro prevention of the emergence of multidrug resistance in a pediatric rhabdomyosarcoma cell line. Clin Cancer Res 2001; 7: 3193–3198.

[7] Cowie FJ, Pritchard-Jones K, Renshaw J, Pinkerton CR. Multidrug resistance modulation in rhabdomyosarcoma and neuroblastoma cells. Int J Oncol 1998; 12: 1143–1149.

[8] Delerba P, Frascella E, Mascino B, Mandruzzato S, Zambon A, Rosolen A, Carli M, Ninfo V, Zanovello P. MAGE, BAGE and GAGE gene expression in human rhabdomyosarcomas. Int J Cancer 2001; 93: 85–90.

[9] Eralp Y, Bavbek S, Basaran M, Kaytan E, Yaman F, Bilgic B, Darendeliler E, Onat H. Prognostic factors and survival in late adolescent and adult patients with small round cell tumors. Am J Clin Oncol 2002; 25: 418–424.

[10] Fernández JE, Concha A, Aránega A, Ruiz-Cabello F, Cabrera T, Garrido F. HLA I and II expression in rhabdomyosarcomas. Immunobiology 1991; 182: 440–448.

[11] Fernández JE, Prados J, Melguizo C, Arena N, Malavasi F, Álvarez L, Aránega A. Characterization of a new human embryonal rhabdomyosarcoma cell line, RMS-GR. Jpn J Cancer Res 1998; 89: 525–532.

[12] Ferrandis E, DA Silva J, Riou G, Benard I. Coactivation of the MDR1 and MYCN genes in human neuroblastoma cells during the metastatic process in the nude mouse. Cancer Res 1994; 54: 2256–2261.

[13] Harris PE, Lupu F, Hong B, Reed EF, Suciu-Foca N. Differentiation stage-specific self-peptide bound by major histocompatibility complex class I molecules. J Exp Med 1993; 177: 783–790.

[14] Hennequin E, DelVincourt C, Pourny C, Jardillier JC. Expression of mdr 1 gene in human breast primary tumors and metastases. Breast Cancer Res Treat 1993; 26: 267–274.

[15] Hirose M, Hamano S, Tobinaik K, Kuroa Y. Cytosidal activity of PBL, LAK and IDEC-C2B8 and expression of HLA class 1, ICAM-1, and CD20 in vincristine-resistant hematologic cell lines. J Hemother 1999; 22: 237–244.

[16] Kerb R, Hoffmeyer S, Brinmann U. ABC drug transporter: hereditary polymorphisms and pharmacological impact of MRP1 and MRP2. Pharmacogenomics 1991; 2: 51–64.

[17] Krishna R, Mayer LD. Multidrug resistance (MDR) in cancer. Mechanism, reversal using modulators and the role of MDR modulators in influencing the pharmacokinetics of anticancer. Eur J Pharm Sci 2000; 11: 265–283.

[18] Levine A, Holzmayer TA, Roninson IB, Das Gupta TK. MDR 1 expression in metastatic malignant melanoma. J Surg Res 1993; 54: 621–624.

[19] López-Nevot M, Cabrera T, De La Higuera B, Ruiz-Cabello F, Garrido F. Production and characterization of monoclonal antibodies against leukemic cells. Inmunologia 1986; 5: 51–59.

[20] Masci AM, Scala S, Racioppi L, Zappacosta S. Cell surface expression of major histocompatibility class I antigens is modulated by P-glycoprotein transporter. Human Immunol 1995; 42: 245–253.

[21] Melguizo C, Prados J, Fernández JE, Vélez C, Alvarez L, Aránega A. Actinomycin D causes multidrug resistance and differentiation in a human rhabdomyosarcoma cell line. Cell Mol Biol 1994; 40: 137–145.

[22] Moulton TA, Jiang H, Guarini L, Fetell MR, Fisher PB. Induction of growth suppression and modification of gene expression in multidrug-resistant human glioblastoma multiforme cells by recombinant human fibroblast and immune interferon. Int J Cancer 1992; 51: 373–378.

[23] Neefjes JJ, Momburg F, Hammerling GJ. Selective and ATP-dependent translocation of peptides by the MHC-encoded transporter. Science 1993; 261: 769–777.

[24] Oda Y, Rose I, Radig K, Wagemann W, Mittler U, Roessner

A. Expression of MDR1/p-glycoprotein and multidrug resistance-associated protein in childhood solid tumours. Virchows Arch 1997; 430: 99–105.

[25] Prados J, Melguizo C, Marchal JA, Vélez C, Alvarez L, Aránega A. Therapeutic differentiation in a human rhabdomyosarcoma cell line selected for resistance to actinomycin D. Int J Cancer 1998; 75: 379–383.

[26] Raney RB. Soft-tissue sarcoma in childhood and adolescence. Curr Oncol Rep 2002; 4: 29–298.

[27] Sambrook J, Fristch E, Maniatis T. Extraction and Purification of RNA. In: Molecular Cloning. A Laboratoty Manual (3ª ed). Ed. Cold Spring Harbor Laboratory Press, New York, 2001: 706–712.

[28] Scheper RJ, Bulte JWM, Brakkee JGP, Quak JJ, Van der Schoot E, Balm AJM, Meuer CJ, Broxterman HJ, Kuiper CM, Lankelman J, Pinedo HM. Monoclonal antibody JSB-1 detects a highly conserved epitope on the P-glycoprotein associated with multidrug-resistance. Int J Cancer 1998; 42: 389–394.

[29] Sedlak J, Hunakova L, Sulikova M, Chorvath B. Protein kinase inhibitor-induced alterations of drug uptake, cell cycle and surface antigen expression in human multidrug-resistant (Pgp and MRP) promyelocytic leukemia HL-60 cells. Leuk Res 1997; 21: 449–458.

[30] Shabitts JA, Krishna R, Mayer LD. Molecular and pharmacological strategies to overcome multidrug resistance. Expert Rev Anticancer Ther 2001; 1: 585–594.

[31] Sharma CR, Inoue S, Roitelman J, Schimke TT, Simoni RD. Peptide transport by the multidrug resistance pump. J Biol Chem 1992; 267: 5731–5734.

[32] Weinstein RS, Jakate SM, Dominguez JM, Lebovitz MD, Koukoulis GK, Kuszak JR, Klusens LF, Grogan TM, Saclarides TJ, Roninson IB, Conn JS. Relationship of the expression of the multidrug resistance gene product (P-glycoprotein) in human colon carcinoma to local tumor aggressiveness and lymph node metastasis. Cancer Res 1991; 51: 2720–2726.

[33] Volm M. Multidrug resistance and its reversal. Anticancer Res 1998; 18: 2905–2917.

[34] Zöchbauer S, Schwarzinger I, Strobl H, Lechner K, Pirker R. Relationship between MDR1 gene and surface markers in acute myeloid leukemia. Anticancer Res 1997; 17: 749–752.